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POLLEN ANTAGONISM IN COTTON[1]

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INTRODUCTION

The writers (*24*)[2] have shown that when emasculated flowers of Pima Egyptian (*Gossypium barbadense* L.) or of upland cotton (*G. hirsutum* L.) are pollinated with a mixture of pollen from both types selective fertilization in favor of the like pollen results. The populations resulting from the double pollinations in the earlier experiments (*24, Table II*) yielded the data given in Table 1. From 67 to 82 per cent of the plants were of the type of the female parent, whereas, if there had been no selection among the pollen grains, only 50 per cent of the plants would have been of this type and the others heterozygous (Pima × upland or upland × Pima, F_1). The departure from a 1:1 ratio was very significant in each of the populations; the magnitude of χ^2 indicates, in every case, chances more than 100 to 1 that the departure was not fortuitous.

TABLE 1.—*Evidence of selective fertilization when emasculated flowers of Pima Egyptian and of Lone Star and Acala upland cotton were pollinated with both kinds of pollen, as shown by the percentages of homozygous plants in the resulting populations*

Female parent	Pollens applied [a]	Plants grown	Homozygous plants	χ^2 of departures of numbers observed from numbers expected in the absence of selective fertilization
		Number	*Per cent*	
Pima	Pima and Lone Star	1,280	67.6±0.88	158.6
Lone Star	do	542	77.6±1.21	165.2
Pima	Pima and Acala	682	82.8± .97	293.5
Acala	do	618	67.7±1.27	77.5

[a] In each of the 4 populations, one-half of the flowers were pollinated first with the like pollen and then with the unlike pollen, while on the other half the sequence was reversed. It was thought that combining the subpopulations from the 2 sequences of pollination would give an approximation to the results expected if the 2 pollens had been mixed together in equal quantities before being applied to the stigmas. Since the subpopulations representing the 2 sequences of pollination were of unequal size, instead of basing the percentage of homozygous plants, as given in this table, on the actual number of such individuals in the combined population, the average of the percentages of the 2 subpopulations was taken. In computing the probable error of this average percentage, *n* was taken as twice the number of plants in the smaller of the subpopulations. It is these modified numbers that are given under the heading "Plants grown."

Evidence was given in the paper cited (*24*) that the preponderance of homozygous individuals is not attributable to any lack of compatibility between these types of cotton or to differences in the viability of the two pollens or to selective survival at any stage after fertilization

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[2] Reference is made by number (italic) to Literature Cited, p. 224.

was accomplished. Indications also were obtained that such tubes of the unlike pollen as succeeded in penetrating the ovary had grown as rapidly as the tubes of the like pollen. Further investigation has yielded additional information on these points, which is given in the present paper.

A hypothesis formulated by the writers in attempting to explain the observed facts (*24, p. 339*) assumed that the like pollen stimulates a reaction in the tissues of the pistil, making the latter an unfavorable medium for the development of the unlike pollen. If this assumption is well founded, it follows that the more intimately the two pollens are in contact on the stigmas the greater the selective effect should be. The results of an experiment in which this test was applied are given in this paper. The question as to whether the like pollen must be present in a viable condition in order to exert an inhibiting effect upon the unlike pollen has been investigated also.

It has been shown by Jones (*21*) that in maize the degree of selective fertilization depends in large measure upon the degree of consanguinity of the two forms, usually being greatest between forms that are most distantly related. Experiments to determine whether this is the case in cotton, also, are described in this paper.

The experimental work was done at the United States field station, Sacaton, Ariz. In all experiments the flowers were emasculated in the evening before the day of anthesis and were pollinated the following morning. For information concerning the structure and ontogeny of the cotton flower, in relation to pollination and fertilization, the reader is referred to an earlier publication by one of the writers (*22, Pl. I–IV, VI, and VII*).

Pollen grains of the two types used in these experiments, Pima Egyptian and upland cottons, are shown in Figure 1.

POSSIBLE ALTERNATIVE EXPLANATIONS OF OBSERVED PHENOMENA

It is obvious, of course, that effects similar to those of selective fertilization would result from pollinations with mixed pollen if there were a difference in the compatibility of the two forms involved, or if the two pollens differed in viability, or if there were selective survival in favor of one or the other class of zygotes. Furthermore, selective fertilization might occur in the absence of any mutually antagonistic action of the two pollens if the rate of growth of the tubes differed consistently. The conclusion was reached that none of these explanations applied to the results previously published. This conclusion is supported by the results of the investigations reported herein.

CONDITIONS PRODUCING EFFECTS SIMILAR TO THOSE OF SELECTIVE FERTILIZATION

Relative Compatibility of the Pollens as Indicated by Degrees of Fertilization

Evidence was given in an earlier paper by one of the writers (*22, Table 23*) that pollen of upland cotton, when present alone on the stigmas of Pima Egyptian cotton flowers, effected fertilization in a degree equal or even superior to that effected by Pima pollen when present alone on the Pima stigmas. Additional data as to the mutual compatibility of these cottons have been obtained in later experiments, the results of which are summarized in Table 2.

TABLE 2.—*Mutual compatibility of the two types of cotton as shown by degrees of fertilization effected by pollen of Pima Egyptian and of upland cotton (Lone Star and Acala varieties) when applied separately to stigmas of one or the other type, measured by the percentage of bolls retained and the mean number of seeds per boll* [a]

Year	Experiment No.	Pollination	Flowers pollinated	Bolls retained	Seeds per boll
			Number	*Per cent*	*Mean number*
1922	1	Pima × Pima	99	99.0±0.67	15.6±0.24
		Pima × Lone Star	100	96.0±1.32	17.0± .20
		Difference		3.0±1.48	1.4± .31
		Lone Star × Lone Star	99	22.2±2.82	30.1±1.14
		Lone Star × Pima	100	12.0±2.19	24.3±1.30
		Difference		10.2±3.57	5.8±1.73
1922	3	Pima × Pima	50	92.0±2.59	15.3± .38
		Pima × Lone Star	50	82.0±3.66	17.6± .36
		Difference		10.0±4.49	2.3± .52
		Lone Star × Lone Star	49	49.0±4.81	33.3± .88
		Lone Star × Pima	50	42.0±4.72	31.5± .69
		Difference		7.0±6.75	1.8±1.12
1925	7	Pima × Pima	93	92.5±1.84	15.6± .22
		Pima × Acala	97	94.8±1.52	16.7± .25
		Difference		2.3±2.39	1.1± .33
1927	2	Pima × Pima	100	65.0±3.22	16.2± .23
		Pima × Acala	100	65.0±3.22	17.5± .24
		Difference		0 ±4.55	1.3± .33
1928	1	Pima × Pima	101	97.0±1.15	18.0± .21
		Pima × Acala	100	93.0±1.72	18.2± .23
		Difference		4.0±2.07	.2± .31
		Acala × Acala	50	98.0±1.33	33.0± .25
		Acala × Pima	50	96.0±1.87	31.1± .51
		Difference		2.0±2.30	1.9± .57

[a] The much heavier rate of boll shedding in upland as compared with Egyptian cottons is reflected in the much lower percentages of bolls retained from pollinations on the upland plants in the experiments of 1922. The effect of removal of most of the early flowers is seen in the high percentage of bolls retained on the Acala plants in the experiment of 1928. The mean number of seeds per boll is, of course, much greater in the large 4-lock or 5-lock upland bolls than in the small, mostly 3-lock, Pima bolls.

The data in Table 2 show no significant differences for either cotton in the percentages of bolls retained from pollination with like and with unlike pollen. As indicated by this criterion, the two pollens are equally efficient when applied separately to the stigmas of either type. In all the experiments except that of 1928, in which there was practically no difference, the mean number of seeds in the Pima bolls was significantly greater when the flowers were pollinated with the unlike (upland) pollen than when they were pollinated with the like (Pima) pollen. In the three experiments in which the results of pollinations on upland plants were compared, the mean number of seeds in the upland bolls was greater when the flowers had been pollinated with the like (upland) pollen than when they had been pollinated with the unlike (Pima) pollen, and the difference was significant in two of these experiments.

So far as may be concluded from the data at hand, it appears that on the Pima stigmas the fertilizing ability of the unlike (upland) pollen usually is somewhat greater than that of the like (Pima) pollen and

that on the upland stigmas there is usually a difference in favor of the like (upland) pollen. As a result of these differences, the action of selective fertilization in favor of the like pollen would be partly counteracted in the Pima flowers and somewhat exaggerated in the upland flowers. Somewhat greater viability of the upland pollen seems to be indicated by these comparisons, although, as will be shown in the following section, tests in sugar solution have shown no consistent difference in the viability of the two kinds of pollen.

Relative Viability of the Pollens as Indicated by Behavior in Sugar Solutions

No very satisfactory artificial medium for the germination of cotton pollen has been devised (*2*). Observation of the germination of the pollen when placed on the stigmas of cotton flowers is difficult because the pollen tubes can not be distinguished readily from the hyaline projections of the stigmatic surface. It was discovered by A. E. Longley [3] that pollen of Pima and of upland cotton germinates readily when placed on corn silks, although care must be taken not to have the surface too moist, as otherwise the grains burst. Doctor Longley noted that both on corn silks and in an artificial medium of 2 per cent agar+15 per cent cane sugar the tendency to bursting is greater in Acala upland pollen than in Pima pollen. He also observed that the tubes from the smaller upland pollen grains are only about half the diameter of the Pima pollen tubes and grow much more rapidly than the latter in this medium.

Observation during several seasons at Sacaton, Ariz., has shown that upland cottons produce much nonviable pollen during the early part of the summer and during periods of high humidity, while throughout the season the pollen of Pima cotton usually contains but a small proportion of nonviable grains. This difference would have seriously impaired the results of experiments in selective fertilization had not the precaution been taken of deferring such experiments until observation indicated that the viability of the two pollens had become approximately equal.

Tests of pollen viability have been made in a 5 per cent solution of cane sugar, in which medium, and also in distilled water, the grains explode suddenly instead of germinating slowly as they do when placed on cotton stigmas. The term "pseudogermination" was applied by Andronescu (*1*) to this sudden extrusion of the contents of the grain (*22, p. 22–25*). It seems justifiable, however, to regard the proportion of the total number of grains in the field of the microscope that extrude their contents in these media as an indication of the relative viability, because it is the abnormally small and the abnormally large grains that fail to explode.

In the earlier experiments the viablity of the two pollens, Pima and upland, was tested by the method just described (*24, p. 332*), and no significant difference was detected. Similar tests were made in connection with some of the experiments described in the present paper and will be considered in relation thereto. No indications were obtained of differences in viability of such magnitude as to impair the evidence in favor of selective fertilization.

[3] Unpublished work.

Differential Survival of Homozygotes and Heterozygotes

The criterion of selective fertilization used in all the experiments has been the percentage of homozygous (like × like) individuals in the resulting population. This criterion would be open to serious objection if there were differential survival of either class of plants at germination or in some later stage of growth. If the homozygous individuals survived in relatively greater number, the effect would be the same as that of selective fertilization in favor of the like pollen. On the other hand, better survival of the heterozygous plants would counteract the effect of selective fertilization in favor of the like pollen or, if the difference in rate of survival were great enough, it might create the appearance of selective fertilization in favor of the unlike pollen.

Determinations of weight and of percentage of germination were made on the seeds resulting from separate application of the two pollens in experiment 1 of 1922. (Table 2.) The results, summarized in Table 3, indicate that the heterozygous seeds, obtained by fertilization with unlike pollen, were somewhat heavier and germinated better. The difference in percentage of germination was of considerable magnitude between the seeds from upland flowers pollinated with like and with unlike pollen, respectively. It may be assumed, therefore, that whatever differential survival occurs at germination favors the heterozygotes and prevents the full expression of selective fertilization in favor of the like pollen.

Table 3.—*Relative germination of seeds obtained by pollinating flowers of Pima Egyptian and of Lone Star upland cotton with pollen of each type separately*

Pollination	Mean weight of 50 seeds [a]	Mean germination
	Grams	*Per cent*
Pima × Pima	6.44±0.03	85.9±1.08
Pima × upland	6.76± .03	91.0± .86
Difference	.32± .04	5.1±1.38
Upland × upland	6.29± .05	57.2±2.11
Upland × Pima	6.76± .20	74.4±1.86
Difference	.47± .21	17.2±2.81

[a] The weights were determined on lots of 50 seeds each, distributed among the several pollinations as follows: Pima × Pima, 30; Pima × upland, 32; upland × upland, 13; upland × Pima, 5. The means were computed from the weights of the several lots representing each pollination.

In connection with the experiments on selective fertilization performed in 1922 and described in an earlier publication, consideration was given to the question whether there had been differential survival at any stage from germination to the time when the plants were large enough to distinguish with certainty between the homozygotes and the heterozygotes. The conclusion was reached that selective survival at or after germination, as indicated by comparing the percentages of hybrids where the conditions were more favorable and less favorable to the growth of the plants, had not been an important factor in bringing about the observed results. Indirect evidence was presented that the results are not attributable to selection between the homozygotes and heterozygotes at any stage between fertilization and germination (*24, p. 333–335*).

At the time these experiments were performed the soil of the Sacaton station was only very locally and nowhere seriously infested

with nematodes. Subsequently, however, pronounced infestation took place. It has been found at this station that cotton of the Egyptian type is much more susceptible than upland cotton to injury by these organisms, which frequently cause heavy mortality among the plants of Pima and other Egyptian varieties, especially in the seedling stage. It has been observed, also, that the Pima × upland F_1 plants are less susceptible to such injury than the Pima plants, although more so than the upland plants. There is good reason to believe that selective survival of the heterozygous plants, due to this factor, has invalidated some of the results obtained in recent years, as will be discussed in connection with the experiments involved.

DIFFERENTIAL GROWTH RATE OF POLLEN TUBES AS A CAUSE OF SELECTIVE FERTILIZATION

The occurrence in cotton of selective fertilization in favor of like pollen is believed to have been proved by the evidence that differences in compatibility and viability of the pollens and rate of survival do not account for the observed excess of homozygous plants in the populations from mixed pollinations. The question next to be considered is whether the selection is due to differential growth rate of the pollen tubes.

Evidence has been obtained by Jones and other investigators (*21 p. 10–34*) that in maize and other plants selective fertilization is due to differences in the rate of development of the two pollens, conditioned, presumably, by genetic differences in the determiners for pollen-tube growth. Direct observation of growth of the pollen tubes in the pistils of the cotton plant has not been attempted, but two indirect methods of calculating the relative rates of growth have been employed. The first method involved the pollination of different individual flowers, some with like and some with unlike pollen. The pistils were excised at the summit of the ovary at stated intervals after the pollen was deposited, and the degrees of fertilization attained in each period with each kind of pollen were compared. This will be referred to hereafter as the excision method.[4]

The second method, used by Correns in his studies of selective fertilization in Melandrium (Lychnis), assumes that in plants whose ovaries contain many ovules vertically superposed, if two pollens are applied that differ in their rate of tube growth, the faster growing pollen will fertilize the upper ovules first and if the quantity of pollen is limited the lower ovules will be left to fertilization by the slower-growing kind. If the resulting seeds from the upper and the lower half of the capsule are planted separately, a higher percentage of hybrids from the lower seeds than from the upper seeds would therefore indicate that the tubes of the unlike pollen had grown more slowly than the tubes of the like pollen, and vice versa.[5]

[4] This method was used in studies of Oenothera by Heribert-Nilsson (*18, 19*) and of Melandrium by Correns (*8*). The term "certation" (Zertation) was used by Heribert-Nilsson to designate differential growth rate of the pollen tubes. Harland (*14*) suggests the term "agonisis," meaning a struggle, for this phenomenon.

[5] Correns (*8, p. 338–341*) pollinated flowers of a recessive white-flowered race of *Melandrium rubrum* Garcke (*Lychnis dioica* L.) first with pollen of a dominant red-flowered race and several hours later with pollen of the white-flowered race. The seeds from the upper and the lower part of the resulting capsules were planted separately and there was a much higher percentage of red-flowered individuals in the population from the upper seeds than in the population from the lower seeds. He concluded that the pollen tubes that enter the ovary first tend to fertilize the upper ovules. A similar method was used by Hiorth (*20*) in studies of differential pollen-tube growth in Oenothera.

Growth Rate Tested by Excision Method

pollens applied separately

In an experiment (No. 3) performed in 1922, flowers on plants of Pima and of Lone Star upland cotton were emasculated in the evening and pollinated the following morning, some with the like and some with the unlike pollen, giving four different pollinations: Pima × Pima, Pima × upland, upland × upland, and upland × Pima. The flowers representing each pollination were then divided into lots of approximately 50 each, and the styles and stigmas of the several lots were removed, by excision at the summit of the ovary, at successive intervals. The excisions were made at 5, 8, and 11 p. m. of the day of pollination and at 5 a. m. of the day following, corresponding, respectively, to the following number of hours after the pollen was applied: 8, 11, 14, and 20. In an approximately equal number of flowers representing each pollination the styles and stigmas were not excised, and these flowers served as controls. The number of flowers of a given pollination, excised at a given hour or left without excision, was in no case fewer than 44. The bolls developing from these several treatments were collected when they matured, and record was made of the number of bolls from each lot of flowers and of the number of seeds in each boll. From these data the statistical constants in Table 4 were computed.

The results from excision of the styles and stigmas at successive intervals, as given in Table 4, show that while the ovaries of a few of the Pima flowers had been penetrated within 8 and 11 hours after pollination by enough pollen tubes to insure retention of the boll, no bolls were retained by the upland flowers excised at these intervals. This indicates a slower rate of growth of the pollen tubes in the upland pistils, especially as the average length of the latter above the summit of the ovary is only about 60 per cent of the average length of the corresponding portion of the Pima pistils. Even when excision was deferred until 14 hours after pollination, the percentage of bolls retained, relative to that of the respective controls, was much greater from the Pima than from the upland flowers; but the number of seeds per boll, relative to that of the respective controls, indicated better fertilization of the upland than of the Pima flowers during this period.

A comparison of the two pollens as to relative growth of their tubes in the pistils of each kind of cotton shows that the rate of penetration of the ovary, as measured by the fertilization attained, was nearly the same for both pollens, in both the Pima and the upland flowers. Neither the percentage of bolls retained nor the mean number of seeds per boll differed significantly between the two pollinations on flowers of either type, with three exceptions. Bolls matured from 2 of the 49 Pima flowers pollinated with Pima pollen and excised after 8 hours, whereas no bolls matured from the upland flowers pollinated with Pima pollen and excised at the same time. Also, in bolls from Pima flowers excised after 11 hours and from those not excised, pollination with upland pollen gave a significantly greater mean number of seeds than pollination with Pima pollen.[6] So far as may be judged by the

[6] When comparison is made of the results from excision after 14 hours with the results from the unexcised (control) flowers the relative mean number of seeds after 14 hours is seen to be considerably greater from the pollination Pima × Pima than from the pollination Pima × upland. As percentages of the numbers from the respective controls, the values are 56.2 ± 4.1 and 39.7 ± 4.3. The probable errors were computed as the probable error of the quotient $\frac{\text{Mean number of seeds from 14-hour excision}}{\text{Mean number of seeds from control}} \times 100$. The difference, however, is hardly significant, being only 2.8 times its probable error.

results obtained by this method, there is, however, no important and consistent difference in the rate of growth of the tubes of the two kinds of pollen in the pistils of either type of cotton.

TABLE 4.—*Relative completeness of fertilization, as indicated by the percentage of bolls retained and the mean number of seeds per boll, in flowers of Pima Egyptian and Lone Star upland cotton, pollinated with like and with unlike pollen, from which the styles and stigmas had been removed by excision at successive intervals or had not been removed* [a]

[Experiment No. 3, 1922]

Interval between pollination and excision	Pollination	Bolls retained	Seeds per boll
		Per cent	*Mean number*
8 hours	Pima × Pima	4.1±1.89	14.0±2.86
	Pima × upland	0	0
	Difference	4.1±1.89	14.0±2.86
	Upland × upland	0	0
	Upland × Pima	0	0
	Difference		
11 hours	Pima × Pima	14.0±3.31	4.9±1.58
	Pima × upland	10.0±2.86	13.2±1.84
	Difference	4.0±4.40	8.3±2.42
	Upland × upland	0	0
	Upland × Pima	0	0
	Difference		
14 hours	Pima × Pima	60.4±4.77	8.6± .59
	Pima × upland	54.0±4.75	7.0± .74
	Difference	6.4±6.74	1.6± .95
	Upland × upland	8.3±2.68	27.2±3.35
	Upland × Pima	14.0±3.31	25.3±1.87
	Difference	5.7±4.26	1.9±3.84
20 hours	Pima × Pima	76.0±4.07	15.0± .57
	Pima × upland	78.0±3.95	16.3± .45
	Difference	2.0±5.68	1.3± .73
	Upland × upland	15.9±3.73	32.1±1.59
	Upland × Pima	18.8±3.81	32.9±1.30
	Difference	2.9±5.34	.8±2.06
Control (not excised)	Pima × Pima	92.0±2.59	15.3± .38
	Pima × upland	82.0±3.66	17.6± .36
	Difference	10.0±4.48	2.3± .52
	Upland × upland	49.0±4.81	33.3± .88
	Upland × Pima	42.0±4.72	31.5± .69
	Difference	7.0±6.74	1.8±1.12

[a] The heavier rate of boll shedding, characteristic of upland as compared with Pima cotton, is indicated by the much lower percentages of bolls retained from the upland flowers in comparison with those retained from the Pima flowers. The mean numbers of seeds per boll are of course greater in the large 4-lock and 5-lock upland bolls than in the small, mostly 3-lock Pima bolls.

Great variation in the rate of growth of individual pollen tubes is indicated by the data in Table 4. In a few of the Pima flowers the number of tubes that penetrated the ovary within 8 hours after pollination was sufficient to insure retention of the boll, but 20 hours were required to effect a degree of fertilization approximating that of the control flowers. Taking 33 mm. as the average length of the Pima

pistils from the summit of the stigmas to the summit of the ovary, the indicated variation in the mean hourly growth rate of the pollen tubes was from 1.65 mm. (33/20) to 4.12 mm. (33/8). In the upland flowers a sufficient number of pollen tubes to insure retention of the boll had not penetrated the ovary within 11 hours after pollination. Assuming that at least 12 hours is required for penetration, and taking 20 mm. as the average length of the Lone Star upland pistils, the most rapid growth rate of the pollen tubes in the upland cotton pistils apparently did not exceed 1.67 mm. (20/12) per hour, which is practically the same as the minimum computed for the rate of growth in Pima pistils.[7]

TABLE 5.—*Relative completeness of fertilization, as indicated by the percentage of bolls retained and the mean number of seeds per boll, in flowers of Pima Egyptian cotton, pollinated with like and with unlike pollen, from which the styles and stigmas had been removed by excision at successive intervals or had not been removed*

[Experiment No. 7, 1925]

Pollination	Bolls retained and mean number of seeds after indicated interval between pollination and excision					
	12 hours		21 hours		Control (not excised)	
	Bolls retained	Seeds	Bolls retained	Seeds	Bolls retained	Seeds
	Per cent	*Mean number*	*Per cent*	*Mean number*	*Per cent*	*Mean number*
Pima × Pima	23.5±2.88	5.0±0.67	81.2±2.69	13.3±0.30	92.5±1.84	15.6±0.22
Pima × upland	27.3±3.02	5.8± .40	82.6±2.58	15.4± .28	94.8±1.52	16.7± .25
Difference	3.8±4.17	.8± .78	1.4±3.73	2.1± .41	2.3±2.39	1.1± .33

In a similar experiment (No. 7), performed in 1925, flowers on Pima plants were emasculated, and half of them were pollinated with Pima pollen and half with pollen of Acala upland cotton. In approximately equal numbers of flowers of each pollination the styles and stigmas were excised at intervals of 8, 10, 12, and 21 hours after the pollen was applied; a fifth lot was left without excision, as a control. Each lot comprised from 93 to 100 flowers. There was no fertilization of the flowers excised 8 hours after pollination, and the number of bolls retained from flowers excised after 10 hours was only 2 in the population Pima × Pima and 5 in the population Pima × upland. The results from the later excisions and from the controls are given in Table 5.

[7] The length of the pistil from the summit of the stigmas to the apex of the ovary was measured at Sacaton in 1922 on 50 flowers each of Pima Egyptian and Lone Star upland cotton and in 1923 on 50 flowers each of Pima and of the Lone Star and Acala varieties of upland cotton. The means obtained were as follows (millimeters):

	Pima	Lone Star	Acala
1922	32.2±0.16	17.2±0.11	.....
1923	34.1± .11	22.6± .17	23.4±0.15
Average	33.2	19.9	.....

Determinations of the distance from the apex of the ovary to the uppermost ovule gave the following means: 2.9 ± 0.03 mm. for Pima (15 flowers) and 2.3 ± 0.08 mm. for Lone Star (10 flowers). Since in the experiments described in this section the excisions were made at the apex of the ovary, only the distance from the summit of the stigmas to that point need be considered.

Under natural conditions the time available for penetration of the ovary is limited by the number of hours from the opening of the corolla and deposition of pollen to the abscission of the pistil at the summit of the ovary when the flower withers. The mean length of this period, as determined at Sacaton in 1922 on 50 Pima flowers, was 29 hours. It has been observed that it is somewhat longer in humid, cloudy weather and shorter in dry, sunny weather.

The percentages of bolls retained from the two pollinations did not differ significantly in any of the subpopulations, but from flowers of which the styles were excised 21 hours after pollination and from the unexcised (control) flowers the mean number of seeds was significantly higher in bolls from flowers pollinated with unlike pollen (Pima × upland) than in bolls from flowers pollinated with like pollen (Pima × Pima). The mean differences were 5.1 and 3.3 times their respective probable errors. These differences suggest a difference in the viability of the two pollens. Tests in sugar solution of the pollens used in this experiment indicated that such was the case, as the pollen from several Pima flowers contained from 20 to 50 per cent of defective grains, while the number of such grains in the samples of upland pollen was negligible. There was, however, no appreciable difference in the rapidity and completeness of explosion of the normal grains of the two pollens. The pollinations were made with such a large excess of pollen grains that it is improbable that the content of defective grains in the Pima pollen was an important factor in the result.

TABLE 6.—*Differences between Pima and upland cotton in rate of pollen-tube growth, as indicated by the increase in mean number of seeds per boll from the control flowers excised later or not at all over the mean number of seeds per boll from flowers excised earlier*

Year	Experiment No.	Pollination	Interval between pollination and excision		Increase in mean number of seeds in the controls
			Shorter	Longer	
			Hours	*Hours*	
1920	[a]5	Pima × Pima	16½	24½	2.9±0.78
		Pima × upland	16½	24½	.8±1.06
		Difference			2.1±1.32
1922	[b]3	Pima × Pima	14	(c)	6.7± .70
		Pima × upland	14	(c)	10.6± .82
		Difference			3.9±1.08
1922	[b]3	Upland × upland	14	(c)	6.1±3.46
		Upland × Pima	14	(c)	6.2±1.99
		Difference			.1±4.00
1925	[d]7	Pima × Pima	12	(c)	10.6± .71
		Pima × upland	12	(c)	10.9± .47
		Difference			.3± .85

[a] The data of this experiment are given in another paper (*22*, *Table 24*). No flowers were left unexcised, so flowers excised 24½ hours after pollination are taken as the controls.
[b] Data given in Table 4 of this paper.
[c] Not excised.
[d] Data given in Table 5 of this paper.

Table 6, summarizing the results of several experiments, shows a comparison of the degrees of fertilization attained by applying the two pollens separately and allowing a shorter and a longer interval for the pollen tubes to penetrate the ovary. The data indicate in only one comparison a significant difference between the two types of cotton in the rate of growth of the pollen tubes. In the experiment of 1922 the increase in the mean number of seeds from the unexcised

control flowers over the mean number from the flowers excised 14 hours after pollination was decidedly greater for the Pima flowers fertilized with upland pollen than for the Pima flowers fertilized with Pima pollen. The difference of 3.9 seeds between the mean increases may be considered significant, being 3.6 times its probable error. In this case the fact that fertilization was more nearly complete after 14 hours in the flowers that received pollen of their own variety than in the flowers that received pollen of another species (upland) indicated more rapid growth of the tubes of the like pollen.

It may not be concluded, however, from the evidence in Table 6 that more rapid growth of the tubes of the like pollen in the Pima pistils is the rule. In the experiment of 1920 the comparison indicated more rapid growth of the unlike pollen, although the difference was not significant and in the experiment of 1925 there was practically no difference between the two pollens. The only comparison of the rate of growth of the tubes of different pollens in pistils of upland cotton (upland × upland and upland × Pima in the experiment of 1922) gave no evidence of a difference in rate of growth of the like and the unlike pollen tubes.

POLLENS APPLIED MIXED

It has been seen that excision experiments in which the two kinds of pollen were applied separately gave no evidence of a consistent difference in the growth rate of the tubes. It does not follow, however, that the growth rates are the same when a mixture of both pollens is presented to the stigmas of the same individual flower. To test this point, an experiment was made in 1925 in which Pima flowers were emasculated in the evening and pollinated the following morning at 9 a. m. with an intimate mixture of approximately equal quantities of Pima pollen and Acala upland pollen. The pistils of equal numbers of these flowers were then excised at the summit of the ovary at intervals of 9, 11, 13, 20, and 24 hours, respectively, after pollination; a sixth lot was left unexcised, as a control.

Tests in sugar solution of pollen from five flowers of each variety, made at the beginning of the experiment, indicated a slight difference in viability in favor of the upland pollen. The estimated percentage of small and doubtless defective grains varied from 5 to 10 per cent in the several Pima flowers and from 2 to 5 per cent in the several upland flowers. In each lot of pollen there were also a few abnormally large grains, which failed to explode or exploded only after very long immersion. There appeared to be no difference between the Pima and upland pollens in the rapidity and completeness of explosion of the normal-sized grains.

This experiment was planned on the assumption that if the tubes of the like (Pima) pollen reached the ovary sooner than the tubes of the unlike (upland) pollen, the percentage of homozygous individuals in populations grown from the resulting seeds would be greater in populations from the earlier-excised flowers than in populations from flowers excised later or not excised. Only 2 or 3 bolls each matured from the lots of flowers that were excised 9 and 11 hours after pollination. The seeds produced by the remaining lots of flowers were planted in 1926, and the percentages of homozygous individuals in these populations are given in Table 7.

TABLE 7.—*Percentages of homozygous (Pima) plants in populations from seeds produced by Pima flowers which were pollinated with a mixture of Pima and upland pollens and from which the styles and stigmas had been removed by excision 13, 20, and 24 hours after pollination, or had not been removed*

[Experiment No. 6, 1925]

Interval between pollination and excision (hours)	Plants grown	Homozygous plants	χ^2 of departures of numbers observed from numbers expected in the absence of selective fertilization
	Number	*Per cent*	
13	83	81.9±2.85	33.8
20	692	88.2± .83	403.8
24	659	90.7± .76	536.6
Control (not excised)	627	87.2± .90	346.8

Contrary to the assumption, the population from the flowers excised earliest contained the lowest percentage of homozygous plants, indicating more rapid growth of the unlike (upland) pollen tubes. The percentage for the 13-hour excision does not, however, differ significantly from any of the others except, possibly, from the percentage for the population from flowers excised after 24 hours, and in this case the difference is only three times its probable error. It must be concluded that this experiment gave no satisfactory evidence of a difference in the growth rate of the two pollens when both are present on the same stigmas.

A very pronounced degree of selective fertilization in favor of the like pollen is shown by the data in Table 7, notwithstanding the somewhat greater viability of the upland pollen indicated by the tests in sugar solution. The departures, in the several populations, from the 50 per cent of homozygous plants expected if there had been no selective fertilization were, respectively, 7, 25, 27, and 23 times the probable error of the departure. The magnitude of χ^2 of the departures of the observed numbers of homozygous and heterozygous plants from the numbers expected had there been no selective fertilization indicates, in every case, chances many more than 100 to 1 that the departure is significant.[8] Since the slight difference in viability of the two pollens indicated by the results of tests in a sugar solution was in favor of the upland pollen and the pollinations were made on Pima flowers, the tendency of this difference would have been to obscure rather than to magnify the effect of selective fertilization.

GROWTH RATE TESTED BY COMPARING POPULATIONS FROM UPPER AND LOWER SEEDS

Another method for determining whether there is differential growth rate of the tubes from like and from unlike pollen is to apply to the stigmas a mixture of the two kinds of pollen and to determine the proportion of homozygous plants in populations grown from the

[8] It is noteworthy that the percentage of homozygous plants in the population from the unexcised (control) flowers in this experiment is almost the same as in the comparable population C of experiment 5 grown the same year from flowers pollinated in the same manner as in the experiment here described, and not excised. (Table 9.)

upper and from the lower seeds in the resulting bolls. This method is based on the assumption that the apical ovules are likely to be fertilized by the pollen tubes that enter the ovary first.[9] In that case the populations would be expected to differ in the percentage of homozygous plants, the percentage being greater in the population from upper seeds if the like pollen grew more rapidly and in the population from lower seeds if the unlike pollen grew more rapidly. In the experiments described in an earlier paper (*24, p. 337, 338*) application of this method showed no significant differences between the populations from upper and from lower seeds, but this may have been due to the fact that the number of pollen grains placed on the stigmas was many times greater than the number of ovules.

With a large excess of pollen, the tendency would be to fertilization of all or nearly all the ovules by the more rapidly growing kind of pollen, and there would be less likelihood of a difference in the proportion of homozygotes and heterozygotes between the populations from upper and from lower seeds. On the other hand, if the number of pollen grains in the mixture does not greatly exceed the number of ovules, fertilization of the upper ovules by the more rapidly growing pollen and of the lower ovules by the slower-growing pollen may be expected.[10] Experiments with pollen mixtures, therefore, were undertaken in which the total number of pollen grains applied to the stigmas was not greatly in excess of the number of ovules.

In an experiment (No. 6) performed in 1926, 15 grains of Pima pollen and 15 grains of Acala upland pollen were mixed together and the mixture was applied to the stigmas of 75 emasculated Pima flowers. Thus, 30 grains of pollen were applied to each flower, although the average number of ovules in the ovary of the Pima flower is approximately 21 (*22, p. 51*). The excess of 9 grains was intended to compensate for possible lack of viability in a few of the pollen grains. Bolls were retained from 62 flowers (82.7 per cent of the number treated) and the mean number of seeds in these bolls was 7.7, whereas in previous experiments in which much larger quantities of pollen were applied to emasculated Pima flowers, the mean number of seeds per boll was from 16.6 to 17.0. It may be concluded that the 30 grains of pollen used in the present experiment were too few to give the maximum degree of fertilization attainable in emasculated and artificially pollinated flowers.

The bolls that matured were divided into upper and lower halves and the seeds from each half were planted separately in 1927 to determine the percentages of homozygous plants. The resulting data, given in the upper section of Table 8, show no evidence of a difference in rate of growth of the two kinds of pollen, the difference between the percentages of homozygous individuals in the two subpopulations having only slightly exceeded the probable error of the difference. Moreover, there was no evidence of selective fertilization,

[9] That this may be the case in cotton is indicated by data obtained by Rea (*29*), who found that in upland cotton bolls the proportion of motes or unfertilized ovules increases from the top to the bottom of the boll.

[10] Correns (*8*) found that in the dioecious *Melandrium rubrum* the percentage of pistillate plants in the resulting progeny was greater when the flowers received a large excess of pollen than when a smaller number of pollen grains was applied. He concluded from this fact that the tubes of the pistillate-determining grains grow faster than the tubes of the staminate-determining grains. References to earlier papers of this author are given in the publication cited. The possibility of distortion of Mendelian ratios as a result of differential growth rate of the tubes of genetically different pollen grains seems first to have been suggested by Renner (*30*), who later (*31*) obtained different ratios in Oenothera depending upon whether few or many pollen grains were used. The assumption was that when pollen is used sparingly the slower-growing tubes have a chance to fertilize more of the ovules.

the percentage of homozygous plants in the combined population not having departed significantly from 50 per cent. The probable reason for the apparent absence of selective fertilization will be considered presently.

TABLE 8.—*Numbers of plants and percentages of homozygous plants in populations grown from seeds from the upper half and from the lower half of bolls produced by Pima flowers pollinated with mixtures of equal numbers of grains of Pima and upland pollen*

EXPERIMENT OF 1926

Pollen grains applied		Plants from—			Homozygous plants from—			χ^2 of departures of numbers observed from numbers expected in the absence of selective fertilization (from all seeds)
Each kind (number)	Total	Upper seeds	Lower seeds	All seeds	Upper seeds	Lower seeds	All seeds	
	Number	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*	
15	30	137	155	292	45.3±2.87	49.7±2.71	47.6±1.97	0.67

EXPERIMENT OF 1927

15	30	272	295	567	57.0±2.02	57.0±1.94	57.0±1.40	11.12
20	40	258	171	429	49.7±2.10	52.7±2.57	50.8±1.63	.11
25	50	78	125	203	50.0±3.82	48.8±3.01	49.3±2.37	.04
30	60	156	182	338	48.7±2.60	47.8±2.50	48.2±1.83	.44

In a similar experiment (No. 1) performed in 1927, four lots of emasculated Pima flowers were pollinated with mixtures of 15, 20, 25, and 30 grains each of two kinds of pollen, Pima and Acala upland, hence with totals of 30, 40, 50, and 60 grains, respectively. There were no significant differences among the several lots of bolls obtained in respect to the mean number of seeds, hence no evidence that 60 grains of pollen effected more nearly complete fertilization than was attained with 30 grains. The mean number of seeds having ranged from 11.9 to 13.3 in the several lots, the degree of fertilization was in all cases much higher than was attained with 30 grains of pollen in the experiment of 1926 but fell considerably short of the degree attained in earlier selective-fertilization experiments in which each flower doubtless received many more than 60 pollen grains.[11]

The seeds obtained by pollination with the several pollen mixtures were planted in 1928 for determination of the percentages of homozygous plants, seeds from the upper and from the lower half of the bolls of each lot being planted separately. The resulting data are given in the lower section of Table 8.

In none of the populations resulting from pollination with different numbers of pollen grains did the subpopulations from upper and from

[11] The better fertilization in 1927 than in 1926 probably is accounted for by the fact that the plants used in the experiment of 1926 were somewhat stunted, while the plants used in 1927 were vigorous and healthy. The deficient fertilization in the experiments of 1926 and 1927 does not necessarily imply that there were not sufficient numbers of viable pollen grains to fertilize more of the ovules, but may have been due to isolated position on the stigmas of the relatively few grains applied. Brink (7) found that in Cucumis the pollen develops better when the grains are massed than when they are scattered. From this fact he inferred the secretion of mutually stimulating substances and concluded that these "are apparently products of the metabolism of the pollen tube; they are readily diffusible and are utilized more completely when the tubes are massed."

lower seeds differ significantly in the percentage of homozygous individuals, the difference in every case being smaller than its probable error. It appears that even when the total number of pollen grains (30) only slightly exceeded the number of ovules, the unlike (upland) pollen fertilized the ovules in the upper part of the ovary as readily as it fertilized the lower ovules. This finding, according with that of the experiment of 1926, indicates that there was not a more rapid growth of the tubes of the like pollen than of the unlike pollen. As the total number of pollen grains was not much greater than the number of ovules, if the tubes of the like pollen (Pima) had penetrated the ovary sooner than the tubes of the unlike pollen there should have been a higher percentage of homozygous plants in the subpopulation from upper seeds than in the subpopulation from lower seeds.

A slight but perhaps significant degree of selective fertilization was shown by the population from pollination with 30 grains, the percentage of homozygous plants being 57 per cent and the departure from 50 per cent being 3.5 times its probable error. The value of χ^2 for the departure from equal numbers of homozygous and heterozygous plants expected in the absence of selective fertilization is 11.1, indicating chances of more than 100 to 1 that the departure is significant. No selective fertilization was shown in the populations from pollination with 40, 50, and 60 grains.

The absence of evidence of a high degree of selective fertilization in the populations of this experiment and in the experiment of 1926 (Table 8) was unexpected. In the experiments of 1922 (Table 1) and in the experiment of 1925 (Table 7) in which each flower must have recived many more than 60 pollen grains, selective fertilization was very pronounced, as the populations grown from seeds produced by double pollination of Pima flowers with approximately equal quantities of Pima and upland pollen contained from 67 to 91 per cent of homozygous individuals. The cause of this difference apparently lies either in the greater viability of the unlike (upland) pollen used in the experiments of 1926 and 1927 or in selective survival in favor of the heterozygotes.

The viability of the two kinds of pollen used in these later experiments was not tested directly, but all earlier tests indicated equal or nearly equal viability; and the two pollens, when applied separately to the stigmas of Pima flowers, have always produced very nearly equal degrees of fertilization. That the survival of the heterozygous plants may have been favored in the experiments of 1926 and 1927 is suggested by the fact that the soil on which these populations were grown had become heavily infested with nematodes and that at Sacaton Pima cotton is much more susceptible than upland cottons to the resulting root-knot disease. The losses from this disease have been particularly heavy in the seedling stage. In 1922 and 1925, when the earlier experiments were performed, nematode infestation was not a factor. It is a fair assumption, therefore, that more of the homozygous (Pima) than of the heterozygous (Pima × upland) plants died from root knot in an early stage of growth; in which case unduly low proportions of homozygous (Pima) individuals would have been recorded. The appearance of a low degree of selective fertilization in favor of the like pollen in only one of the populations from the experiment of 1927 may have been due to the chance location of this population in less heavily infested soil.

The fact that in the experiments of 1926 and 1927 with mixtures of the two pollens the subpopulations from upper and lower seeds in no case differed significantly in the percentage of homozygous individuals points to the conclusion that the two pollens were substantially alike in the growth rate of their tubes. Since the excision experiment described in the preceding section also gave negative results as to difference in the rate of growth of the tubes of the two kinds of pollen when both are present on the same stigmas, the conclusion seems warranted that the tubes from such grains of the unlike pollen as escape the inhibiting action of the like pollen grow as rapidly and penetrate the ovary as soon as the tubes of the like pollen. It is inferred that the inhibiting action of the like pollen expresses itself in preventing the germination or early development of many of the unlike pollen grains.

SELECTIVE FERTILIZATION AS AFFECTED BY INTIMACY OF POLLEN MIXTURE AND BY CONDITION OF LIKE POLLEN

It is believed that the evidence here given has established the reality of selective fertilization in favor of the like pollen in cotton and has eliminated differential growth rate of the pollen tubes as a general cause of the phenomenon. How, then, is it to be explained? The hypothesis has been advanced by the writers (*24*) that a reaction takes place in the stigmatic tissue, making this tissue a less suitable medium for germination or development of the unlike pollen. The fact that the unlike pollen when present alone on the stigmas effects fertilization nearly or quite as readily as does the like pollen indicates that the inhibiting substance supposed to be produced in the stigmas develops only under a stimulus supplied by the presence of the like pollen.

If the hypothesis is well founded, the inhibiting action should be greatest when both kinds of pollen are present over the whole stigmatic surface and least when they are placed separately on different parts of the stigmatic surface. In the former case every part of the stigmatic and stylar tissues would be affected by contact with the grains and tubes of the like pollen, while in the latter case tracts of stigmatic tissue not in direct contact with the grains and tubes of the like pollen would be available for penetration by the unlike pollen. It is also essential to an understanding of the problem to know whether the assumed toxin-producing capacity of the like pollen exists only when the pollen cells of the latter are intact and viable. If so, penetration of the stigmas by the pollen tubes would be indicated as a condition of the reaction. Experiments designed to afford information on these points were carried out in 1925, 1927, and 1928.

ANTAGONISM PROPORTIONAL TO INTIMACY OF MIXTURE

An experiment (No. 5) was performed in 1925 to determine whether the degree of antagonism is proportional to closeness of contact of the two pollens on the stigmas. During a period of 10 days (August 7–16), 30 flowers of Pima cotton were emasculated daily and were pollinated with both Pima and Acala upland pollens. Equal numbers of flowers were pollinated in three different ways, as described below.

Treatment A.—Approximately equal quantities of the two pollens were applied to opposite sides of the same stigmas, so that the two pollens were separated as widely as is possible when both were present on the stigmas of the same flower.

The application in this and in treatment B was made by brushing the stigmatic surface with the staminal columns of the pollen-supplying flowers.

Treatment B.—The two pollens were applied by the method used in earlier experiments (*21*), Pima pollen followed by upland pollen on one-half of the number of flowers and the two pollens in the reverse order on the remaining flowers. By combining the resulting subpopulations, the result should approximate that obtained by mixing the two pollens before application to the stigmas. Since both pollens were applied to the whole surface of the same stigmas, they were in closer contact than in treatment A.

Treatment C.—Approximately equal quantities of the two pollens were mixed together thoroughly and the mixture was applied to the stigmas. The numbers of grains of the two kinds of pollens were not counted, but the mixture used on each day of the experiment was examined before it was placed on the stigmas in order to be sure that the two kinds of pollen, readily distinguishable by the size and color of the grains, were present in approximately equal quantities.[12] This method assured the greatest possible intimacy of contact between the two pollens.

A difference in the viability of the two pollens might have invalidated the results. Samples of both pollens, therefore, were tested each day

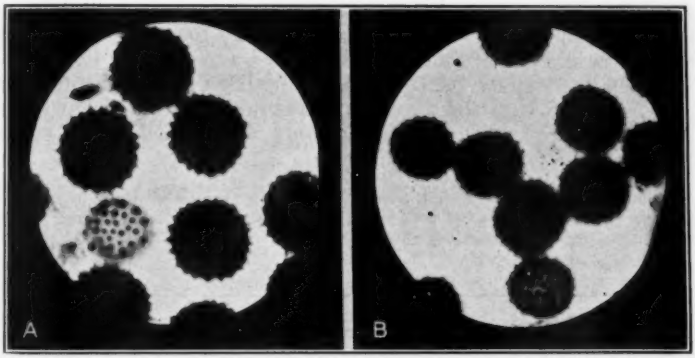

FIGURE 1.—Pollen grains of Pima Egyptian cotton (A) and of Acala upland cotton (B). × 160. The Pima pollen grains are somewhat larger and have somewhat longer spines than the upland grains. The color of the Pima pollen is deep golden yellow, and that of the upland pollen (Acala and Lone Star varieties) is pale cream. The smaller, light-colored grain in each lot of pollen is nonviable. (Photograph by A. E. Longley)

by immersion in distilled water. No appreciable difference in the proportion of grains that exploded was observed. Additional tests, in a 5 per cent sugar solution, were made at the beginning and at the end of the 10-day period of the experiment. The first test showed a decided inferiority of the Pima pollen; the percentage of defective grains ranged from 20 to 50 per cent in the several Pima flowers and was negligible in the Acala flowers. On the other hand, there was no appreciable difference between the two pollens in the promptness and completeness with which the grains of normal size exploded in the sugar solution. In the second test the estimated percentage of small, light-colored, doubtless defective grains (fig. 1) ranged from 5 to 10 per cent in the several Pima flowers and from 2 to 5 per cent in the

[12] Details of the procedure followed in preparing the mixture are as follows: Pima and Acala flowers were collected each morning when the anthers had begun to discharge their pollen and were brought into the laboratory. Approximately equal quantities of each pollen were shaken out on a microscope slide and mixed together thoroughly with a dissecting needle. The mixture was then transferred to gelatin capsules. The mixed pollen was applied by inverting a capsule over the stigmas and revolving it until the stigmas were well covered with pollen. The contents of each capsule served to pollinate two flowers.

several Acala flowers. The varietal difference in the proportion of such grains was therefore much less pronounced than it had been 10 days earlier. No varietal difference in the rapidity and completeness of explosion of the normal-sized grains was detected. In the pollen of both varieties, in addition to the very small grains there were a few grains of abnormally large size, which did not explode or did so only after very long immersion.

In spite of the differences shown by these tests in sugar solution, it is believed that the mixtures used in treatment C were not deficient in viable Pima pollen. The proportion of the two kinds of pollen in the mixtures was determined by inspection rather than by count and, since the deep yellow of the viable Pima grains contrasts sharply with the much paler nonviable Pima grains and the nearly colorless Acala upland grains, a marked deficiency of normal Pima pollen in the mixtures could not have escaped notice. It should be noted further that since the pollinations of this experiment were made on Pima flowers an excess of viable upland pollen in the mixtures would have increased the percentage of heterozygous plants in the resulting population and hence would have tended to nullify the effect of selective fertilization in favor of the like pollen.

The seeds obtained by the several methods of pollination were planted in 1926, and the numbers of plants grown and percentages of homozygous (Pima) individuals were determined. The results are given in Table 9.

TABLE 9.—*Number of plants and percentages of homozygous plants in populations from Pima flowers pollinated with both Pima Egyptian and Acala upland pollens, in different degrees of intimacy of mixture*

[Experiment No. 5, 1925]

Treatment	Method of applying the two pollens	Plants grown	Homozygous plants	χ^2 of departures of numbers observed from numbers expected in the absence of selective fertilization
		Number	*Per cent*	
A	Separately, on opposite sides of the stigmas	890	66.6±1.07	98.0
B	Over the whole stigmas but seriatim, Pima followed by upland pollen on half of the flowers, the sequence reversed on the other half, the populations from both sequences as 1 array.*	825	77.1±1.01	242.4
C	Mixed intimately before applying	978	86.4± .74	518.4

* As was the case in the experiment described in an earlier paper (*24, p. 332-333*) the subpopulation from the like + unlike sequence of pollination gave a higher percentage of homozygous individuals (80.3 ± 1.29 per cent) than the subpopulation from the sequence unlike + like (74.1 ± 1.49 per cent). The difference between these percentages is 3.1 times its probable error. As the 2 subpopulations were of somewhat unequal size (431 and 394 plants, respectively) the percentage of homozygous individuals, as given for the whole population, is the average of the percentages of the 2 subpopulations.

A very high and very significant degree of selective fertilization in favor of the like pollen in all three populations is indicated by the data in Table 9. The magnitude of χ^2 of the departures of the observed numbers of homozygous and heterozygous plants from the numbers expected had there been no selective fertilization indicates, in every case, chances many more than 100 to 1 that the departure is significant. It is clear that these results are not attributable to a

difference in the viability of the two pollens, such difference as was observed having been in favor of the unlike (upland) pollen.

A comparison of the results from the several methods of pollination shows that treatment A, in which the two pollens were placed separately on opposite sides of the same stigmas, yielded the lowest percentage of homozygous plants and that treatment C, in which the pollens were mixed together thoroughly before application to the stigmas, yielded the highest percentage. The difference of 19.8 ± 1.30 per cent is highly significant, being 15.2 times its probable error. Treatment B, in which the two pollens were applied on the same stigmatic surface but without being thoroughly mixed, yielded a nearly intermediate percentage of homozygous plants, which differed significantly from the percentages yielded by treatments A and C (differences 7.1 and 7.4 times their respective probable errors).

Since the closeness of contact of the two pollens was least in treatment A and greatest in treatment C, the results of this experiment accord perfectly with the initial assumption that the degree of antagonism should be proportional to the degree of intimacy of contact of the pollens.

ANTAGONISM CONDITIONED BY VIABILITY OF THE LIKE POLLEN

Evidence having been obtained that presence of the like pollen tends to inhibit the development of the unlike pollen and that the magnitude of the resulting effect is proportional to the intimacy of contact of the two pollens, the question arises whether the like pollen exerts this antagonistic action only when present in a viable condition. In an endeavor to answer this question, experiments were performed in 1927 and 1928 in which the viability of the like pollen was destroyed by maceration and immersion in distilled water before it was placed on the stigmas.

Experiment of 1927

Well-grown plants of Pima Egyptian cotton were selected, and on these, during each day of the experiment, flower buds were emasculated and bagged. Other buds on the Pima plants and on plants of Acala upland cotton were merely bagged in order to provide pollen. The following morning some of the bagged but not emasculated Pima flowers were taken to the laboratory, and the pollen was shaken from the anthers into a small agate mortar and thoroughly macerated. The resulting fine paste, mixed with a little distilled water, was carried to the field in a small glass vial. This suspension will be referred to hereafter as "pollen extract." The remaining bagged but not emasculated Pima and upland flowers were taken from the plants and used to provide living pollen. The involucre and corolla of these flowers were excised so that the pollen could be brushed onto the stigmas without previously removing it from the anthers.

The stigmas of half of the emasculated flowers were painted with the suspension of macerated Pima pollen by means of a small camel's-hair brush. Half of the flowers so treated were pollinated with intact Pima pollen (A) and half of them with intact upland pollen (C). The remaining emasculated flowers were used as controls, and their stigmas, not coated with pollen extract, were pollinated likewise, half of them with Pima (B) and half of them with upland pollen (D).

A small tag, on which was written the plant number, date, and a letter indicating the treatment, was attached to each flower as soon as it was pollinated and the flower was then inclosed in a fresh bag to prevent the access of foreign pollen.

The four treatments, given on each of the 10 days of the experiment to equal numbers of emasculated Pima flowers, may be summarized as follows:

(A) Stigmas coated with Pima pollen extract, pollinated with Pima pollen.
(B) (Control) stigmas not coated, pollinated with Pima pollen.
(C) Stigmas coated with Pima pollen extract, pollinated with upland pollen.
(D) (Control) stigmas not coated, pollinated with upland pollen.

A total of 100 Pima flowers received each treatment during the course of the experiment. Each of the bolls obtained, with its tag, was collected as it matured, and the number of seeds was determined. Any seed, however small, that had developed beyond the ovule stage was counted as a fertilized seed. Table 10 shows the percentages of bolls retained from flowers receiving the several treatments and the mean numbers of seeds in these bolls.

TABLE 10.—*Degrees of fertilization attained in Pima cotton flowers of which the stigmas had been coated with macerated Pima pollen and pollinated with Pima pollen (A) and with upland pollen (C) as compared with the control flowers of which the stigmas were not coated but were pollinated with Pima pollen (B) and with upland pollen (D)*

[Experiment of 1927]

Treatment	Pollination of Pima	Flowers treated	Bolls retained	Seeds per boll
		Number	*Per cent*	*Mean number*
A	×Pima, on Pima extract	100	34.0±3.19	11.6±0.63
B	×Pima, control	100	65.0±3.22	16.2± .23
	Difference (B−A)		31.0±4.53	4.6± .67
C	×upland, on Pima extract	100	35.0±3.22	9.6± .47
D	×upland, control	100	65.0±3.22	17.5± .24
	Difference (D−C)		30.0±4.56	7.9± .53
	Difference (D−C)−(B−A)		−1.0±6.43	3.3± .85

Both sets of flowers (A and C) whose stigmas had been coated with pollen extract were much less completely fertilized than the respective controls (B and D), as shown both in the lower percentage of bolls retained and in the smaller mean number of seeds per boll. This lower degree of fertilization doubtless was partly due to the fact that the coating of pollen extract prevented close contact of many of the living pollen grains with the stigmatic surface. It was observed that the pollen adhered much more readily to the untreated stigmas than to those which had been coated with the extract. The bursting of many of the grains when brought into contact with the stigmas that had been moistened by the extract probably was also a factor limiting the fertilization of the treated flowers.[13]

[13] Nearly all the bolls retained by the flowers that had received the extract were from flowers treated on the last five days of the experiment. The reason doubtless was that at first the living pollen was applied immediately after application of the pollen extract. When it was observed that practically no bolls were setting from flowers thus treated the extract was allowed to evaporate on the stigmas before the living pollen was applied. Less than one minute sufficed for this.

If the macerated Pima pollen had had an inhibiting effect upon the capability of the unlike (upland) pollen to accomplish fertilization, then the reduction in the degree of fertilization resulting from application of the pollen extract, as compared with the fertilization of the corresponding control,[14] should have been greater in treatment C than in treatment A. In other words, the difference D − C should be greater than the difference B − A. Table 10 shows that this was not so in regard to percentage of bolls retained, but in mean number of seeds per boll the difference D − C is 3.3 ± 0.86 seeds greater than the difference B − A. The difference between the two differences, being 3.8 times its probable error, may be regarded as significant. The results of this experiment seem to indicate that the inhibiting effect of like upon unlike pollen persists even when the viability of the former presumably has been destroyed by maceration.

In order to ascertain whether the viability of the pollen used in coating the stigmas had been destroyed completely by maceration, the seeds produced by the severally treated flowers were planted in 1928 and the populations were examined on July 19. The population representing treatment A (pollination with Pima pollen on Pima extract) comprised 218 plants, all of which were Pima, as would have been the case, of course, whether or not any of the pollen grains in the extract had remained viable. But if the Pima extract had contained viable pollen, the population representing treatment C (pollination with upland pollen on Pima extract) should have contained at least a few plants from the fertilization Pima × Pima, whereas all of the 283 plants in this population were easily recognizable as Pima × upland F_1. It is probable that even if a few pollen grains escaped maceration by grinding in the mortar they burst promptly and lost their viability when the paste was mixed with distilled water.

TABLE 11.—*Relative survival rate of plants from fertilizations Pima × Pima (treatments A and B) and of plants from fertilizations Pima × upland (treatments C and D), of the experiment of 1927*

Treatments	Pollination	Seed planted	Plants surviving		Nature of the plants
		Number	*Number*	*Per cent*	
A and B	Pima × Pima	1,196	592	49.5±0.97	All Pima.
C and D	Pima × upland	1,136	899	79.1± .81	All Pima × upland F_1.
	Difference			29.6±1.26	

Better survival of the heterozygotes is indicated by the data given in Table 11, which shows that the combined population from flowers pollinated with unlike pollen (C and D) was much larger in proportion to the number of seeds planted than the combined population from flowers pollinated with like pollen (A and B). In earlier experiments, the heterozygotes showed no such marked superiority to the Pima plants in rate of survival,[15] but the soil where these plantings were

[14] It is interesting to note that the mean number of seeds per boll from the control flowers pollinated with upland pollen (D) is significantly greater than the number from the control flowers pollinated with Pima pollen (B), the difference being 3.9 times its probable error. Other examples of increased fertilization of Pima flowers when pollinated with upland pollen are given in Table 2.

[15] In the field plantings of earlier experiments, there appeared to be no superiority of the heterozygotes in this respect, as evidenced by the percentages of heterozygotes in hills containing one or two as compared with hills containing a greater number of plants (*22, p. 46; 24, p. 334*). In laboratory germination tests, however, there were slight differences in favor of the heterozygotes (*22, Table 23*). The results of a similar test, showing better germination of the heterozygous seeds, are given in Table 3 of the present paper.

made in 1928 was heavily infested with nematodes, and it has been observed at Sacaton that the mortality due to these organisms is much heavier in Pima than in upland cotton or in Pima × upland F_1.

Experiment of 1928

The results of the experiment in 1927 indicated that Pima pollen of which the viability has been destroyed by maceration may inhibit the germination or subsequent development of some of the upland pollen grains on the stigmas of the Pima flowers. The observed effect was slight, however, and it was thought advisable to repeat the experiment on a larger scale. This was done in 1928. The number of treatments was increased by using macerated pollen of both Pima and Acala upland and by applying it to flowers of both varieties. The several lots of emasculated flowers of each variety, therefore, were treated as follows:

(A) Stigmas coated with Pima pollen extract, pollinated with Pima pollen.
(B) (Control) stigmas not coated, pollinated with Pima pollen.
(C) Stigmas coated with Pima pollen extract, pollinated with upland pollen.
(D) (Control) stigmas not coated, pollinated with upland pollen.
(E) Stigmas coated with upland pollen extract, pollinated with Pima pollen.
(G) Stigmas coated with upland pollen extract, pollinated with upland pollen.

The first four treatments are the same as treatments A, B, C, and D in the experiment of 1927, while treatments E and G amplify the experiment so as to test the effects of macerated upland pollen also. Treatment B (Pima pollination) served as the control for treatments A and E, and treatment D (upland pollination) served as the control for treatments C and G.

The technic was the same as that of the 1927 experiment, except in two minor details. From the plants selected for treatment all bolls that had set before the beginning of the experiment were removed at the outset, and thereafter all flowers not treated were removed daily. This was done because of Eaton's discovery that removal of many of the flowers and bolls reduces the rate of shedding of those left on the plants (*10*). It was found that the soft, almost flaccid involucres of the upland flowers were much in the way when emasculating and pollinating, a difficulty not experienced in handling the Pima flowers because of the crisper, more rigid texture of the involucre in this type. For this reason the upper portion of the upland involucre was cut away when emasculating the flower bud.

The percentage of bolls retained and the mean number of seeds in these bolls were computed separately for each of the six treatments in each population. The statistical constants thus obtained are given in Table 12. The percentage of bolls retained and the mean number of seeds per boll were much lower from flowers treated with pollen extract (treatments A, C, E, and G) than from the control flowers (treatments B and D). The reason for this difference was given in describing the experiment of 1927.

Table 12 shows also the difference in percentage of bolls retained and in mean number of seeds per boll between each lot from flowers treated with macerated pollen and the lot from the corresponding control flowers. Comparison of the differences between these differences, likewise given in Table 12, should determine whether the macerated like pollen had reduced the degree of fertilization effected by the

unlike pollen. If this had been the case, in the populations from Pima mother plants the difference D − C (upland pollen control minus upland pollen on stigmas coated with Pima pollen extract) should have been significantly greater than the difference B − A (Pima pollen control minus Pima pollen on stigmas coated with Pima pollen extract). Likewise, in the populations from upland mother plants, the difference B − E (Pima pollen control minus Pima pollen on stigmas coated with upland pollen extract) should have been significantly greater than the difference D − G (upland pollen control minus upland pollen on stigmas coated with upland pollen extract).

TABLE 12.—*Number of flowers treated, percentage of bolls retained, and mean number of seeds per boll from emasculated flowers of Pima Egyptian and of Acala upland cotton, some of which had been pollinated with Pima pollen and some with Acala pollen, with and without previous application to the stigmas of macerated pollen (pollen extract) of each type of cotton*

[Experiment of 1928]

PIMA AS MOTHER PLANTS

Treatment	Pollination	Flowers treated	Bolls retained	Seeds per boll *
		Number	*Per cent*	*Mean number*
A	×Pima, on Pima extract	50	74.0±4.18	9.3±0.75
B	×Pima, control	101	97.0±1.15	18.0± .21
	Difference (B−A)		23.0±4.34	8.7± .78
C	×upland, on Pima extract	51	80.4±3.75	9.6± .71
D	×upland, control	100	93.0±1.72	18.2± .23
	Difference (D−C)		12.6±4.13	8.6± .75
	Difference (D−C)−(B−A)		−10.4±6.00	−.1±1.08
E	×Pima, on upland extract	50	72.0±4.28	9.5± .69
B	×Pima, control	101	97.0±1.15	18.0± .21
	Difference (B−E)		25.0±4.44	8.5± .72
G	×upland, on upland extract	50	68.0±4.45	8.2± .59
D	×upland, control	100	93.0±1.72	18.2± .23
	Difference (D−G)		25.0±4.77	10.0± .63
	Difference (B−E)−(D−G)		0 ±6.52	−1.5± .96

UPLAND AS MOTHER PLANTS

A	×Pima, on Pima extract	50	44.0±4.74	11.6±1.05
B	×Pima, control	50	96.0±1.87	31.1± .51
	Difference (B−A)		52.0±5.10	19.5±1.17
C	×upland, on Pima extract	50	36.0±4.58	14.4±1.43
D	×upland, control	50	98.0±1.33	33.0± .25
	Difference (D−C)		62.0±4.77	18.6±1.45
	Difference (D−C)−(B−A)		10.0±6.99	−.9±1.86
E	×Pima, on upland extract	51	41.2±4.65	10.8± .92
B	×Pima, control	50	96.0±1.87	31.1± .51
	Difference (B−E)		54.8±5.02	20.3±1.05
G	×upland, on upland extract	50	28.0±4.28	14.9±1.57
D	×upland, control	50	98.0±1.33	33.0± .25
	Difference (D−G)		70.0±4.49	18.1±1.59
	Difference (B−E)−(D−G)		−15.2±6.74	2.2±1.91

* Probable errors increased by using Pearson's correction for the standard deviation when the number is small.

Since the hypothesis assumes an inhibiting effect of the like pollen upon the unlike pollen but no such effect of the unlike pollen upon the like pollen, there should have been no significant difference between the pollinations with like and with unlike pollen in the degree to which the fertilization of the flowers of either variety was reduced by the application of macerated pollen of the other variety. In other words, in the populations from Pima mother plants, the difference B − E (Pima pollen control minus Pima pollen on stigmas coated with upland pollen extract) should not have differed significantly from the difference D − G (upland pollen control minus upland pollen on stigmas coated with upland pollen extract). Likewise, in the populations from upland mother plants, the difference D − C (upland pollen control minus upland pollen on stigmas coated with Pima pollen extract) should not have differed significantly from the difference B − A (Pima pollen control minus Pima pollen on stigmas coated with Pima pollen extract).

It is evident from the data in Table 12 that none of these differences between differences, in either percentage of bolls retained or mean number of seeds per boll, is significant with respect to its probable error. In this experiment, therefore, there was no indication that either pollen, when macerated and applied to the stigmas of either variety, exerted an inhibiting action upon the development of the other kind of pollen, except for the purely mechanical effects of the presence of the pollen extract, as noted above.

TABLE 13.—*Classification of populations from the several treatments of flowers on plants of Pima and of Acala upland cotton*

[Experiment of 1928]

SEEDS FROM PIMA MOTHER PLANTS

Treatment	Pollination	Number of plants grown			Expectation if maceration was completely effective (all plants)
		Total	Homozygous	Heterozygous (F_1)	
A	× Pima, on Pima extract	20	20	0	Homozygous.
B	× Pima, control	117	117	0	Do.
C	× upland, on Pima extract	60	0	60	Heterozygous
D	× upland, control	449	0	449	Do.
E	× Pima, on upland extract	33	33	0	Homozygous.
G	× upland, on upland extract	48	0	48	Heterozygous.

SEEDS FROM UPLAND MOTHER PLANTS

A	× Pima, on Pima extract	149	[a] 35	114	Heterozygous.
B	× Pima, control	188	0	188	Do.
C	× upland, on Pima extract	109	109	0	Homozygous.
D	× upland, control	111	111	0	Do.
E	× Pima, on upland extract	124	[a] 18	106	Heterozygous.
G	× upland, on upland extract	33	33	0	Homozygous.

[a] The occurrence of a few homozygous (upland) plants in these 2 populations, where none was expected, is attributable to the fact that the land had been in upland cotton the year before and many plants volunteered from seeds left in the soil.

In order to ascertain whether any error could have arisen through failure to destroy the viability of all the pollen grains in the macerated pollen, the seeds resulting from the several treatments were planted

in 1929 and the populations thus obtained were classified, with the results given in Table 13. Except for the two cases noted in the footnote to the table, of the occurrence of homozygous (upland) plants in populations which should have contained only heterozygous (upland × Pima F_1) plants, the classification indicates complete destruction of the viability of the macerated pollen. The exceptions are attributable to volunteering from seeds left in the soil from the preceding crop. The fact that no heterozygous plants were found in populations that should have been all homozygous warrants the conclusion that the maceration had been effective.

Comparison of the Two Experiments with Macerated Pollen

The experiment of 1927, in which only Pima mother plants were used (see Table 10) showed that in mean number of seeds per boll the difference D − C (upland pollen control minus upland pollen on Pima stigmas coated with Pima pollen extract) was greater than the difference B − A (Pima pollen control minus Pima pollen on Pima stigmas coated with Pima pollen extract), the difference between these differences (D − C minus B − A) being positive in sign and nearly four times its probable error. This result indicated that the treatment with macerated Pima pollen depressed the fertilization of flowers pollinated with the unlike (upland) pollen more than it depressed the fertilization of flowers pollinated with the like (Pima) pollen.

The more comprehensive experiment of 1928, in which plants of both Pima and upland cotton were used as mothers and the effects of extracts of both kinds of pollen were tested, showed, on the contrary, that treatment with like pollen in a macerated condition caused no significant differences in the relative fertilization of flowers of either type whether the pollen supplied in a living state was of the same or of the other type. The presence of the macerated pollen on the stigmas resulted in greatly reduced fertilization, but the action seems to have been purely mechanical, since the reduction was no greater when the living pollen was of the other type than when it was of the same type as the macerated pollen.

The greater weight of evidence, therefore, favors the conclusion that the hypothetical alteration in the stigmatic tissue, unfavorable to the unlike pollen and attributed to action of the like pollen when both kinds are present on the stigmas, takes place only when the like pollen is viable. If such a reaction in the stigmatic tissue is the true explanation of selective fertilization as observed in cotton, presumably the reaction can take place only after the tubes of the like pollen have penetrated the stigmas, a condition that is removed by maceration of the like pollen.

POLLEN ANTAGONISM IN RELATION TO CONSANGUINITY

Jones (*21, p. 75*) found that in maize the degree of selective fertilization in favor of the like pollen is positively and rather highly correlated with the degree of heterosis indicated by the weight of the heterozygous seeds. The coefficient of correlation obtained was 0.50 ± 0.09. In other words, assuming that the greater the degree of heterosis the more remote is the relationship of the two forms that are crossed, it follows that selective fertilization tends to be inversely

proportional to consanguinity. As Jones expressed it, "the somewhat surprising situation exists that in proportion as the cross-fertilization benefits the immediate progeny in its development the less effective is that pollen in accomplishing the union."

In the case of cotton, the writers obtained evidence of a high degree of selective fertilization when two kinds of pollen, each representing a distinct species of Gossypium, were applied to the stigmas of the same flower of one of these species. To ascertain whether the situation is similar to that in maize, experiments involving more nearly related forms were undertaken. Two families of Pima cotton that differ in a single Mendelian character, i. e., full and very weak development of the spot at the base of the petal, afforded suitable material for testing this point, since both parental families were homozygous and the heterozygotes of crosses between them could be distinguished easily from the recessive (weak-spotted) parental form.

EXPERIMENT OF 1924

In 1924, plants of the weak-spotted Pima progeny 3–27–29 were grown at Sacaton adjacent to plants of the full-spotted Pima progeny 13–17–10. Flowers of the former were emasculated and pollinated with both kinds of pollen, taken at random from the several individuals in the respective progeny. The technic was the same as in several of the experiments with Pima and upland cotton, i. e., one half of the flowers were pollinated first with like and then with unlike pollen (like + unlike) and the other half were pollinated first with unlike and then with like pollen (unlike + like).

Equal numbers of flowers received each treatment on each day, and practically the same number of seeds was obtained from each treatment. The bolls that matured from each lot of flowers were halved and the seeds from the upper and from the lower half were kept separate, giving four subpopulations that were grown in 1925. These subpopulations represented: Like + unlike pollination, upper seeds; like + unlike pollination, lower seeds; unlike + like pollination, upper seeds; and unlike + like pollination, lower seeds. Four seeds were planted to the hill, and no thinning was done. Although equal numbers of seeds were planted, the stand varied so greatly in different parts of the plot that the several subpopulations were of very unequal size.

As the plants flowered they were classified. To guard against errors in classification, three flowers on each plant were graded as to the degree of spotting, and the average of the grades of the three flowers was determined. The two classes of plants were easily distinguished, 3.0 representing the maximum average grade in the weak-spotted class and 5.5 the minimum average grade in the strongly spotted class. It may safely be concluded that the former were homozygous, representing the fertilization like × like, and that the latter were heterozygous, representing the fertilization like × unlike.

Table 14 shows the number of plants and percentage of homozygous individuals in the subpopulations as combined in two pairs representing, respectively, the two sequences of pollination and the two positions of the seeds.

The upper section of Table 14 shows that when the like pollen was applied first the percentage of homozygous individuals in the resulting

population was considerably in excess of the 50 per cent expected had there been no selective fertilization. The departure amounts to 10.4 per cent and is four times its probable error. The more advantageous position of the pollen applied first on the stigmas may partly account for this excess (*24, p. 332, 333*), but if this had been the only factor there should have been a corresponding excess of heterozygous plants in the population from flowers that received the unlike pollen first. In fact, however, there was a small but not significant excess of homozygous plants in the latter population.[16]

TABLE 14.—*Number of plants and percentage of homozygous plants in the several populations resulting from pollination of flowers of a homozygous weak-spotted family of Pima cotton with pollen of both the weak-spotted family and of a homozygous full-spotted family of the same variety*

[Experiment of 1924]

Population based on—	Plants grown	Homozygous plants
Sequence of pollination:	*Number*	*Per cent*
Like + unlike (all seeds)	321	60.4±1.84
Unlike + like (all seeds)	234	53.8±2.20
As one array (all seeds)	555	[a] 57.1±1.42
Position of seeds in boll:		
Upper seeds (both sequences)	245	62.4±2.09
Lower seeds (both sequences)	310	53.9±1.91

[a] Because of the difference in size of the subpopulations from the like + unlike sequence of pollination (all seeds) and the unlike + like sequence (all seeds) the percentage for the whole population (555 individuals) was not computed directly from the total number of plants and of homozygous individuals but was taken as the average of the percentages of the subpopulations representing the 2 sequences of pollination.

The average of the percentages of homozygous individuals in the two subpopulations (from like+unlike and from unlike+like pollinations) should approximate the result that would have been obtained if equal quantities of the two kinds of pollen had been mixed together before application to the stigmas. The average, given in Table 14 as the percentage of the whole population, shows an excess of 7.1 per cent of homozygous plants, and the departure from the 50.0 ± 1.42 per cent expected had there been no selective fertilization in favor of the like pollen is 3.5 times its probable error. The departures of the observed numbers of homozygotes and heterozygotes from the equal numbers expected if there had been no selection among the pollen grains give a value for χ^2 of 11.2, indicating chances of more than 100 to 1 that the departures are significant.

The question whether the tubes of the like pollen grew faster than the tubes of the unlike pollen is also of interest. If such were the case, there should have been a higher percentage of homozygous individuals in the population from seeds in the upper part of the boll than in the population from seeds in the lower part of the boll. The data given in the lower section of Table 14 indicate that this was the case, the percentage of homozygous individuals being 8.5 ± 2.83 per cent greater from the upper than from the lower seeds. The difference is only three times its probable error, hence doubtfully significant,

[16] The departure in the subpopulation from unlike+like pollination from the expectation (60.4 per cent heterozygotes), if sequence of pollination had been the only factor and had had the same effect as in the like+unlike population, is 141 (60.4 per cent of 234)−108 (the actual number heterozygous)=33±5.05. The probable error of this departure was computed as

$$0.6745 \times \sqrt{\text{The expected per cent } (0.604) \times 1 - \text{the expected per cent } (0.396) \times n \ (234)}.$$

but the fact that it is of nearly the same magnitude as the excess in percentage of homozygous individuals in the whole population suggests that the small degree of selective fertilization shown in this experiment may have been due to the more rapid growth of the tubes of the like pollen. It is possible, therefore, that these families of Pima cotton differ genetically in their determiners for rate of pollen-tube growth.

EXPERIMENT OF 1925

The experiment of 1924 was repeated the following year on material from the same homozygous Pima families, weak-spotted and full-spotted. The pollinations were made in 1925, and the resulting seeds were planted in 1926. The technic was in all respects similar to that of the first experiment except that the upper and lower seeds were not separated. The results are given in Table 15.

TABLE 15.—*Number of plants and percentage of homozygous plants in the populations resulting from pollination of flowers of a homozygous weak-spotted family of Pima cotton with pollen of both the weak-spotted family and a homozygous full-spotted family of the same variety*

[Experiment of 1925]

Sequence of pollination	Plants grown	Homozygous plants
	Number	*Per cent*
Like + unlike	507	54.8 ±1.49
Unlike + like	524	36.1 ±1.41
As one array	1,031	[a] 45.45±1.05

[a] Average of the percentages of the 2 populations.

In this experiment the sequence in which the two pollens were applied made a much larger and much more significant difference between the resulting percentages of homozygous individuals than was the case in the experiment of 1924, indicating that the stigmas in the later experiment may have been more densely covered with the pollen applied first in the sequence unlike + like than in the earlier experiment. The average of the percentages of the two subpopulations in the present experiment differs from that of the earlier experiment in showing a deficit (45.45 ± 1.05) instead of an excess (57.1 ± 1.42) of homozygous individuals. (See Tables 14 and 15.) The departure from the 50 per cent expected if there had been no selective fertilization is only three times its probable error, but the deviations from equal numbers of homozygous and heterozygous individuals give a value for χ^2 of 8.75, indicating chances of more than 100 to 1 that the deviations were significant.

The two experiments, therefore, although alike in the technic and material employed, gave opposite results. A small degree of selective fertilization is indicated by both experiments, but in the first experiment the selection was in favor of the like pollen and in the second experiment it was in favor of the unlike pollen. As the matter stands at present, it may be concluded that these two families of the same species and variety of cotton are so closely related that there is very little, if any, antagonism between their pollens when present together on the same stigmatic surface. So far as they go, the results are in

harmony with those obtained by Jones (*21*) in maize, indicating that selective fertilization diminishes with increasing consanguinity of the two forms.

HYPOTHESIS OF POLLEN ANTAGONISM

It has been demonstrated by the writers that when pollens of both Pima and upland cotton are applied in approximately equal quantities to the stigmas of emasculated flowers of either type the resulting progeny shows a marked preponderance of plants from fertilization by like pollen (Pima × Pima or upland × upland). In other words, unlike pollen is at a disadvantage in fertilizing the flowers when like pollen also is present on the stigmas, as shown in Tables 1, 7, and 9.

If there were any lack of compatibility between the two types of cotton, these results would require no further explanation, but there is abundant evidence, summarized in Table 2, of nearly perfect compatibility when either pollen is applied alone to stigmas of the other type. This nearly or quite complete mutual compatibility of Pima and upland cotton is surprising in view of the fact that they belong to very distinct species, representing each of the main groups of cultivated American cottons. Pima (*Gossypium barbadense*) is of the South American group and upland (*G. hirsutum*) is of the Mexican group (*23, p. 207, 208*). These types differ in many morphological characters and also in such physiological characters as rate of boll shedding (*25, p. 652, 653*) and selective absorption of certain components of the soil solution (*15, 16*).

A pronounced difference in the viability of the pollens also would account for the observed facts, but repeated tests in vitro have given no evidence of differences sufficient to account for the observed inequalities in the proportion of homozygous and heterozygous plants shown by the populations from mixed pollinations.

Differential survival, after fertilization, in favor of the homozygotes would have the same effect as selective fertilization in favor of the like pollen, but in all cases where differences have been observed in the rate of survival, during or after germination, the advantage has been with the heterozygotes. Such differential survival as has occurred in the experiments, therefore, has been of a nature to obscure rather than to magnify the effect of selective fertilization in favor of the like pollen.

It is also possible that there may have been selective survival among the zygotes immediately after fertilization, resulting in the death at a very early stage of a disproportional number of zygotes from like × unlike unions. The nearly perfect compatibility of the two cottons and the greater vigor of the heterozygotes at and after the stage of germination make this improbable, but the possibility should not be ignored. Comparison of the mean number of seeds with the mean number of ovules, assuming that all undeveloped ovules represent unsuccessful heterozygous unions, should indicate whether this assumption may account for the observed preponderance of homozygous plants in the adult population. Computation on this basis, in five populations in which there was a marked indication of selective fertilization, increased the percentage of possible heterozygous individuals from 32.4 to 47.0, from 17.2 to 35.3, from 11.8 to 23.7, from 9.3 to 20.0, and from 12.8 to 21.4. In all but one of these populations the

deficit of heterozygous plants is still far too great to warrant the assumption that selective survival rather than selective fertilization is the explanation.[17]

The evidence seems conclusive that the observed facts may be interpreted only on the basis of selection between the two kinds of pollen grains. Difference in the rate of growth of the pollen tubes is the explanation that suggested itself first.

It is obvious that if two kinds of pollen differ consistently in this respect, the faster-growing pollen will have the advantage in fertilizing the ovules when both kinds are present on the stigma of the same flower. Evidence obtained by Jones and others and reviewed by Jones (*21, p. 6–34*) indicates that in maize, Oenothera, Melandrium, Datura, and other plants the two kinds of male gametes produced by a heterozygous individual may differ in the rapidity with which they effect contact with the female gametes, and that as a result there is selective fertilization and a consequent distortion of expected Mendelian ratios. In these cases it appears that the difference is of a genetic nature and that frequently the gene determining the rate of growth of the tube is linked with a gene for some visible character. As expressed by Brieger (*3, p. 187*), "Mendelian factors exist which produce a selection among the gametes of a plant heterozygous for these factors, and both the mating of like with like or of unlike with unlike may be favored." Jones also found evidence (*21, p. 72–73*) that the pronounced selective fertilization in favor of the like pollen, observed by him in maize when a mixture of two kinds of pollen was applied to the same stigmas, was caused, at least in part, by the more rapid growth of the tubes of the like pollen.

Is selective fertilization in cotton to be explained on this basis? The evidence obtained by pollination of flowers of Pima and of upland cotton with pollen of both types was negative. When the pollens were applied separately and the styles and stigmas were excised at successive intervals, consistently greater fertilization, at a relatively short interval after pollination, was not effected by the like pollen as compared with the unlike. When the pollens were applied mixed there was not a greater proportion of homozygous plants in the population from flowers of which the styles had been excised comparatively soon after pollination than in the population from flowers in which, by postponing or omitting the excision, a longer period had been afforded for penetration of the ovary by the pollen tubes. Finally, comparison of the populations from seeds in the upper and in the lower part of the capsules obtained by pollinating flowers with mixed pollen did not show a higher percentage of homozygous plants in the population from upper seeds, as should have been the case, according to the results of Correns's experiments with Melandrium (*8*), if there had been more rapid growth of the tubes of the like pollen.

It may be concluded, therefore, (1) that selective fertilization between Pima and upland cottons really occurs, the excess of homozygous individuals in the populations from mixed pollination not being attributable to lack of mutual compatibility, different viability

[17] Moreover, the assumption that all undeveloped ovules represent unsuccessful heterozygous unions is unwarranted, since, even when the flowers are pollinated with like pollen only and in abundant quantity, some of the ovules always fail to develop. The data in Table 2 indicate that when Pima flowers were fertilized with Pima pollen the mean number of seeds in the resulting bolls in no case exceeded 18, whereas the mean number of ovules in the Pima ovary is approximately 21.5.

of the pollens, or selective survival at any stage after union of the male and female gamete, and (2) that differential growth rate of the pollen tubes is not the explanation, such of the tubes of the unlike pollen as develop being able to penetrate the ovary as rapidly and to accomplish fertilization as readily as the tubes of the like pollen. It would seem that the phenomenon is essentially different from selective fertilization in Zea, Oenothera, and Melandrium, which is attributed to genetic differences in the rate of pollen-tube growth. The writers, therefore, have chosen the term "pollen antagonism" to designate the supposed cause of selective fertilization as observed in cotton.

The only hypothesis of pollen antagonism that seems applicable was stated in an earlier publication, as follows (*24, p. 339*):

> * * * the presence of like pollen in some way prevents the germination or subsequent development of many of the unlike pollen grains when both kinds are present on the stigmas. That the inhibiting factor does not reside in the stigmas themselves when like pollen is absent seems clear from the fact that when applied separately the unlike pollen is not inferior to the like pollen in rapidity of development and ability to effect fertilization. It is conceivable, however, that the presence of pollen of the same type may induce a physiological reaction in the stigmas which makes them a relatively unfavorable medium for the germination or growth of pollen of a different type. The further assumption must be made that, in spite of this unfavorable condition, some of the unlike pollen grains are able to accomplish fertilization, possibly because they are more resistant, possibly because they happen to be so placed as to avoid the tracts of stigmatic tissue affected by contact with the like pollen.

The results of experiments described in the present paper are believed to support this hypothesis. It is shown (Table 9) that the degree of selective action is proportional to the intimacy of mixture of the two pollens. The excess of homozygous individuals in the resulting population was least when the two pollens were deposited separately on opposite sides of the stigmas and greatest when an intimate mixture of the two pollens was applied to the whole stigmatic surface. This finding favors the assumption that the development of the unlike pollen tubes is less hindered in tracts of stigmatic and stylar tissue not immediately in contact with the tubes of the like pollen. Evidence also was obtained (Table 12) that the ability of the like pollen to inhibit the development of the unlike pollen is lost when the viability of the former is destroyed by maceration, from which it is inferred that the tubes of the like pollen must penetrate the stigmas in order to produce the inhibiting reaction.

A reaction of some kind within the stigmatic tissue is suggested by the fact that the results of the pollinations with mixed pollen in the earlier experiments (summarized in Table 1) showed the selective fertilization in favor of the like pollen to be of the same order of magnitude, whether the two pollens were applied to Pima or to upland cotton flowers. To account for the phenomenon on the basis of a direct toxic action of the one kind of pollen on the other would require the improbable assumption that the Pima pollen is toxic to the upland pollen only when the two kinds happen to be present on Pima stigmas and that upland pollen is toxic to Pima pollen only on the upland stigmas. The fact that there was selective fertilization in favor of the like pollen resulting from treatment A of the experiment of 1925 (Table 9) in which the two kinds of pollen grains, although present on

the same stigmas, were not in contact, is further evidence against a direct toxic action of the one kind of pollen on the other.[18]

In seeking an explanation of a very different phenomenon, self-sterility as observed in Nicotiana, East and Park (*9*) inferred the occurrence of a chemical reaction in the pistil induced by pollen, in this case by the unlike pollen. They wrote (*9, p. 363–364*):

> These results appear to us to show that the pollen tubes in a selfed pistil are not inhibited in their growth by substances secreted in that pistil, but rather that a substance or substances are secreted in the pistil after a compatible cross which accelerate growth, and that the direct cause of this secretion is a catalyzer which the pollen-tube nucleus is able to produce because the zygotic constitution of the plant producing it is different in certain particular hereditary factors from that of the plant on which it is placed * * *. The action must be local, because the presence of compatible pollen tubes does not accelerate the growth of self pollen tubes.

That the assumed reaction in the pistil of the cotton plant also must be local is indicated by the fact that the selective effect was least when the two pollens were deposited separately on opposite sides of the stigmas.

Yasuda (*35*) sought to determine whether the "Linienstoffe," as Correns termed the substances assumed by him to control compatibility, are secreted in the style or in the ovary. He performed an ingenious experiment, involving two self-incompatible strains of Petunia, in which styles of each strain were grafted on ovaries of each strain, giving four combinations, with intact pistils of each strain as controls. It was found that the growth rate of the tubes of both kinds of pollen was determined by the identity of the ovary and not of the style in the artificial systems. Yasuda concludes that the Linienstoffe are secreted by the ovary and diffuse thence into the style.[19]

No evidence as to the nature of the inhibiting substance supposed to be produced in the pistil of the cotton plant is now available. Biochemical tests of extreme delicacy probably would be required to detect it. It is conceivable that the substance acts so as to render ineffective one or more of the enzymes present in the pollen grains and requisite for penetration of the tissues of the pistil and for utilization of the reserve food stored in them.[20]

A hypothesis advanced by Swingle (*33*) in explanation of metaxenia, or direct effect of pollen on tissues of the mother plant, is of interest in this connection. Swingle suggests that a hormonelike substance, secreted after fertilization by the embryo or the endosperm

[18] The phenomenon, therefore, differs from that observed in animals by Godlewski (*11*), who found that eggs of a sea-urchin could be fertilized by sperm of a worm, but if the sperms of both animals were mixed together, both lost their ability to fertilize the sea-urchin eggs. Results similar to Godlewski's, from experments in vitro with plant pollen, are reported by O'Connor (*27*), who found that development of the tubes of one kind of pollen was checked or inhibited in the presence either of pollen or of extracts of the stigmas and other tissues of a plant belonging to another species, genus, or family. He concluded (*27, p. 480*) that the inhibiting substances probably are amino compounds and that "in anglosperms, each species contains within each cell substances which are toxic to foreign pollen."

[19] It would be difficult to explain pollen antagonism as observed in cotton on the basis of an ovarian secretion unless the selection between the two kinds of pollen becomes operative only after their tubes have entered the ovary. Otherwise it would be necessary to assume that a stimulus initiated by the tubes of the like pollen as they penetrate the stigmas is transmitted downward to the ovary, causing production of a secretion which, diffusing up through the style, acts unfavorably upon the development of the tubes of the unlike pollen.

[20] Paton (*28*) investigated the pollen of 18 species of plants and detected 10 different enzymes, of which, including pectinase, were present in all the pollens. Green (*12, p. 409*) found that "the style itself contains enzymes to assist in preparing the reserve materials for absorption by the pollen tube, while the latter excretes the same ferments during its progress down the conducting tissue." The same investigator also discovered that "when the pollen grain has lost the power of germinating, the quantity of diastase has materially decreased." The complexity of the chemistry and physiology of pollen has been brought out in studies by Brink and by other investigators whose work he reviews (*4, 5, 6, 7*).

and diffusing into adjacent tissues of the mother plant, produces certain alterations in the latter. Metaxenia, discovered in the date palm, has been found by one of the writers (*17*) to occur in cotton also.[21]

Pollen antagonism seems more analogous to anaphylactic than to hormone reactions in animal bodies. The action of the like pollen may be compared to that of an antigen, stimulating the production of antibodies in the tissue into which it is introduced. The analogy is far from perfect, because, in the case under consideration, the pollen presumably acting in the manner of an antigen is genetically like the body in which the reaction is supposed to take place, and the resulting "antibody" is supposed to attack the foreign pollen.[22]

There is as yet little evidence of the occurrence in plants of substances comparable to hormones and antibodies, but the extensive occurrence and great importance in the animal kingdom of hormone effects and of anaphylactic reactions makes it reasonable to suppose that analogous substances and reactions occur in the vegetable kingdom.[23] The discovery of such phenomena as metaxenia and pollen antagonism suggests that biochemical research in this field may prove fruitful.

SUMMARY

Emasculated flowers of Pima and of upland cotton, pollinated with approximately equal quantities of pollen of both types, have shown a marked degree of selective fertilization; the resulting populations have contained a much higher percentage of homozygous than of heterozygous plants.

Application of either pollen separately showed that these cottons are highly compatible, as fertilization of the flowers of either Pima or upland cotton has been effected almost or quite as readily by the unlike as by the like pollen.

So far as could be determined by observation and by tests in media not suitable for normal germination, there were no differences in the viability of the two pollens that could account for the selective action observed.

No evidence was obtained of selective survival at any stage after formation of the zygote that would explain the preponderance of homozygous plants in the populations. Selective survival undoubtedly occurred in some of the experiments, owing to infestation of the soil with nematodes, which are known to cause much greater mortality among the Pima plants than among the Pima × upland F_1 plants. In all such cases survival of the heterozygotes was favored, so this

[21] The presence of hormonelike substances in plants had been suggested previously by Haberlandt (*13*, p. 41), who considered that the results of his experiments on cell division in wound tissues demonstrated the existence of what he terms "division hormones," supposed to be secreted by cells of the leptome tissue, and "wound hormones," secreted by the injured cells themselves. He did not ascertain the chemical nature of the assumed substances but suggested that they may be amines.

[22] Wells (*54*, p. 705) states that "as a general rule, the more closely related the animal furnishing the antibodies is to the one furnishing the antigen the less antigenic activity or antibody response will be obtained." He adds, however, "Some proteins * * * may be so foreign to the blood stream and the active tissues of the body that they incite antibody formation when introduced into the blood stream of even the same animal from which they came."

[23] Antibody production has been suggested as an explanation of immunity to diseases in resistant races of plants, but positive evidence apparently is lacking. Kostoff (*26*, p. 75) summarizes as follows the results of experiments in which various species of Solanaceae were grafted one on another: "* * * mutual induction of antibodies in scion and in stock was found. The acquired immunity in such plants was tested by precipitin reactions. The induced antibodies were specific in certain species." Silberschmidt (*52*) criticizes certain details of the methods used by Kostoff and concludes that the results of his own experiments do not indicate the occurrence of "acquired" precipitins in plants to the extent that antibodies occur in animals.

factor tended to nullify rather than to accentuate the appearance of selective fertilization.

Comparisons of the degrees of fertilization effected when the styles were excised at shorter and at longer intervals after pollination and of populations grown from seeds in the upper and in the lower half of the boll gave no consistent evidence of a differential rate of growth of the tubes of the like and of the unlike pollen. This factor, apparently responsible for selective fertilization as observed in Zea, Oenothera, and other plants, does not account for the situation met with in Gossypium.

The only explanation that seems tenable is that the presence of the like pollen induces a reaction in the stigmatic tissues of such nature as to render them less suitable for the development of the unlike pollen. Apparently the effect is extremely local or else individual pollen grains differ greatly in their ability to withstand the unfavorable condition, since in all the experiments with pollen mixtures some of the ovules were fertilized by the unlike pollen.

Evidence of the localization of the reaction was afforded by an experiment in which the like and unlike pollens were (1) deposited separately on opposite sides of the same stigmas; (2) mixed, but not intimately, and applied to the whole surface of the stigma; and (3) mixed intimately and applied to the whole surface. The percentage of homozygous plants was least in the population from treatment 1 and greatest in the population from treatment 3.

Experiments in which the viability of the like pollen was destroyed by maceration before it was applied to the stigmas gave conflicting results, but the weight of the evidence favors the conclusion that the inhibiting effect upon the unlike pollen takes place only when the like pollen is intact and viable. Therefore, penetration of the stigmas by the tubes of the like pollen seems requisite to the setting up of the reaction. This supports the assumption that the inhibiting substance is produced in the stigmatic or stylar tissue in response to a stimulus supplied by the tubes of the like pollen.

If the hypothesis is well founded, the phenomenon observed in cotton is of a chemical or physiological nature. The term "pollen antagonism" is suggested in order to distinguish it from the selective fertilization observed in other plants and attributed to differential growth rate of the pollen tubes, conditioned by specific genes determining the rates of growth.

Little or no selective fertilization was observed between more nearly related forms, these being two families of Pima cotton differing only in a simple Mendelian character. The evidence is too scanty, however, to warrant the conclusion that the degree of pollen antagonism in cotton is definitely related to the degree of consanguinity, as Jones found to be the case with selective fertilization in maize.

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LIGHT INTENSITY IN RELATION TO PLANT GROWTH IN A VIRGIN NORWAY PINE FOREST [1]

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INTRODUCTION

One of the vital problems confronting foresters in the United States is that of securing a vigorous stand of young trees to replace those removed in logging. Any cutting necessarily changes the conditions for plant growth in the forest. The forester is concerned with the type of cutting which will prove most favorable for the establishment and growth of a new stand.

The virgin forest offers an ideal place to study the effects of various factors on forest reproduction, because in it can be found trees of various ages, openings of different sizes, and many densities of upper canopies; in addition, an ample seed supply is usually present. It should be possible, therefore, from a careful study of conditions in virgin stands, to gain much information on the requirements of the young growth and on what might be expected from various types of cutting. It was with this idea in mind that the writer undertook a study of light conditions and forest growth in a virgin Norway pine forest.

The present study does not attempt to take into consideration all factors which affect plant growth, but rather to take measurements of a single factor and to see how these measurements may be correlated with the vegetation present. As shown by Adams (*1*),[3] air temperature, soil temperature, soil moisture, relative humidity, and evaporation are all changed by thinning a forest stand. All of these factors, as well as light intensity, are more or less directly correlated with solar radiation. If, therefore, within any climatic and edaphic unit area a single factor is to be chosen for correlation with forest growth, light would seem to be the most promising.

Forest trees, however, as shown by Toumey (*16*) and others, tend to deplete soil moisture more rapidly than less massive forms of vegetation. It should be borne in mind, therefore, that root competition for both moisture and nutrients is an important concomitant factor to be taken into account in determining the significance of the light data obtained in this study. In this connection, however, it may be mentioned that preliminary results in the Lake States indicate that an overstory tends to protect the undergrowth from severe drought injury.

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[1] Received for publication July 25, 1931; issued April, 1932. "Light," in this paper, is used synonymously with solar radiation unless specifically qualified.

[2] The writer wishes to acknowledge his indebtedness to Mary C. Shirley, who assisted him with the vegetation enumeration in the field and with the editing of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 244.

The common and scientific names of trees, shrubs, and herbs mentioned in this article are as follows:[4]

TREES

Common Name	Scientific Name
Red maple	*Acer rubrum* L.
Paper birch	*Betula papyrifera* Marsh.
Jack pine	*Pinus banksiana* Lamb.
Norway pine	*Pinus resinosa* Soland.
Northern white pine	*Pinus strobus* L.
Aspen	*Populus tremuloides* Michx.
Pin cherry	*Prunus pennsylvanica* L. f.
Red Oak	*Quercus borealis* Michx. f.
Bur oak	*Quercus macrocarpa* Michx.

SHRUBS

Alder	*Alnus rugosa* (Du Roi) Spreng and *A. incana* (L.) Moench.
Thicket shadblow or June berry	*Amelanchier oblongifolia* (Torr. and Gray) Roem.
Bearberry	*Arctostaphylos uva-ursi* (L.) Spreng.
Inland Jersey-tea	*Ceanothus ovatus* Desf.
Bunchberry	*Cornus canadensis* L.
American hazelnut	*Corylus americana* Walt.
Beaked hazelnut	*Corylus rostrata* Ait.
Dwarf bush-honeysuckle	*Diervilla lonicera* Mill.
Wintergreen	*Gaultheria procumbens* L.
American twinflower	*Linnaea borealis* L., var. *americana* (Forbes) Rehder.
Appalachian cherry	*Prunus susquehanae* Willd. (syn. *P. cuneata* Raf.).
Chokecherry	*Prunus virginiana* L.
Rose	*Rosa* sp.
Red raspberry	*Rubus idaeus* L., var. *aculeatissimus* (C. A. Mey.) Reg. and Til.
Willow	*Salix* spp.
Lowbush blueberry	*Vaccinium angustifolium* Air. (syn. *V. pennsylvanicum* Lam.).

HERBS

PTERIODOPHYTES

Groundcedar	*Lycopodium complanatum* L.
Bracken	*Pteridium aquilinum* (L.) Kuhn, syn. *Pteris aquilina* L.

MONOCOTYLEDONS

Sedge	*Carex* sp.
Bluebead	*Clintonia borealis* (Ait.) Raf.
Grass	*Gramineae* sp.
Mayflower	*Unifolium canadense* (Desf.) Greene, syn. *Maianthemum canadense* Desf.

DICOTYLEDONS

American wood anemone	*Anemone quinquefolia* L.
Pussytoes or everlasting	*Antennaria* sp.
Spreading dogbane	*Apocynum androsaemifolium* L.
Wild sarsaparilla	*Aralia nudicaulis* L.
Smooth aster	*Aster laevis* L.
Bigleaf aster	*Aster macrophyllus* L.

[4] Nomenclature follows Sudworth (*15*) for trees and American Joint Committee on Horticultural Nomenclature (*1*) for other plants.

Common Name	Scientific Name
Aster	*Aster* sp. incerf.
Late aster	*Aster tardiflorus* L.
Harebell	*Campanula rotundifolia* L.
Common pipsissewa	*Chimaphila umbellata* (L.) Nutt.
Bastard toadflax	*Comandra pallida* A. DC.
Erect bindweed	*Convolvulus spithamaeus* L.
Trailing-arbutus	*Epigaea repens* L.
Fireweed	*Chamaenerion angustifolium* (L.) Scop., syn. *Epilobium angustifolium* L.
Virginia strawberry	*Fragaria virginiana* Duchesne.
Northern bedstraw	*Galium boreale* L.
Cream pea vine	*Lathyrus ochroleucus* Hook.
Puccoon	*Lithospermum canescens* (Michx.) Lehm.
Narrowleaf cowwheat	*Melampyrum lineare* Lam.
Fringed polygala	*Polygala paucifolia* Willd.
Rattlesnake-root	*Prenanthes alba* L.
Sidebells pyrola	*Pyrola secunda* L.
Roundleaf pyrola or shinleaf	*Pyrola americana* Sweet.
Dwarf dewberry	*Rubus triflorus* Richards.
Goldenrod	*Solidago* sp.
American germander	*Teucrium canadense* L.
Early meadowrue	*Thalictrum dioicum* L.
American starflower	*Trientalis americana* (Pers.) Pursh.
Cow vetch	*Vicia cracca* L.
Violet	*Viola conspersa* Reichenb.

RELATED INVESTIGATIONS

Studies on the growth of plants exposed to different intensities of light indicate that growth is almost directly proportional to the amount of light available up to values of about 700 foot-candles of artificial light or about 50 per cent of full sunlight in latitudes of the northern United States. Intensities higher than 50 per cent sometimes cause a decrease in growth, especially in plants whose natural habitat is in the shade (*12*). Shantz (*11*), working in Louisiana, found that several plants produced maximum growth with 15 per cent of full sunlight, while others required 50 per cent or more. Zillich (*17*), in Germany, found that several weeds produced best growth in 33 per cent light; cultivated plants, on the other hand, produced maximum dry weight in 100 per cent light and showed a marked decrease when shaded. Many others have studied the influence of shading on plant growth and similarly reached the conclusion that slight shading is sometimes beneficial, whereas heavy shading invariably causes a decrease in growth. The amount of shading required to obtain the best growth varies with the latitude and climatic conditions of the station in question.

The effect of shade in natural forest stands has also been studied, but the results are somewhat less consistent. Moore (*8*) established a series of seed spots in dense shade and in small openings in four forest types of Mount Desert Island, Me. He sowed Norway and northern white pines and white and red spruce seed in prepared spots. Survival was poor for all species in the shade, except in the northern hardwood type. Growth and vigor of the seedlings were in every case markedly better in the small openings.

Pearson (*9*, *10*) studied the growth and survival of western yellow pine in the southwestern United States, with particular reference to the influence of light. He found that after a good seed year, seedlings would come up even more abundantly on the bare areas under the

clumps of trees than in the openings. Practically all of those in dense shade died, however, or became unthrifty within four or five years. Even on the north side of tree groups survival was poor and annual height growth was only 0.4 inch to 3 inches, as against 3 to 6 inches for seedlings of the same age in adjacent openings.

Gast (*5*), during an entire growing season, measured the total radiation received at three different stations where cuttings had recently been made. These measurements were correlated with the growth of the leaders of white pine saplings. He found the leader growth to be apparently directly proportional to the radiation, up to the intensity of full sunlight. The average leader growth of white pine trees 11 years old under a canopy transmitting 27 per cent light was only 2 inches per year and showed little increase with increasing age of the trees. He believes this to represent about the minimum radiation intensity for growth of white pine. Since Gast's measurements of sunlight were made by a continuously recording mechanism operating throughout the summer, his percentage values are higher than those of most workers who make readings only on bright days.

Holch (*7*) grew bur oak, red oak, hickory, linden, and walnut on three forest sites—the prairie, a bur oak forest, and a linden forest. The light values averaged 10.4 per cent for the oak and 3.5 per cent for the linden station on the basis of the prairie station as 100 per cent. The growth of both roots and shoots was greatest for all species in the prairie and least in the linden forest, where most of the plants died before the end of the third season. Photosynthesis was very rapid in all species at the prairie station, was weak at the bur oak station, and very weak at the linden station. Evaporation, transpiration, and soil and air temperatures decreased with decreasing light, and soil moisture increased. Soil moisture was, however, sufficient in all three sites. Under the conditions of Holch's experiment, survival, growth, and photosynthesis were directly correlated with light and inversely correlated with available water content of the soil.

Grasovsky (*6*) placed northern white pine, Norway pine, hemlock, red oak, and chestnut oak seedlings in boxes with a window in one end. At the end of 10 months those so far removed from the window that they received no more than 300 foot-candles of illumination during the entire period were still alive and apparently in vigorous condition. No measurements of dry weight were made to determine whether the plants had actually grown. Grasovsky states that only a moderate increase over the minimum light-intensity requirement for survival was necessary to maintain growth and that thereafter the effect of added light on growth was not at all proportional to the intensity. Measurements of the light intensity under white pine canopies showed it to be in all cases in excess of the minimum light requirements of the plants tested. He concludes that the intensity of light reaching the forest floor is not the limiting factor in accounting for the presence or absence of forest reproduction in the fully stocked stands where the investigation was conducted. His conclusions are somewhat at variance with those cited above. The investigations, however, were carried out under conditions sufficiently different to account for any discrepancies in results.

Atkins and Poole (*3*) studied the correlation between light intensities and plant distribution in an old garden. In the deepest shade they found only straggling branches of English ivy which tended to

give way to enchanter's nightshade when the light increased some sixfold. Various other plants were associated with the higher light values. There seemed to be a very definite tendency for the vegetation to change with changing light conditions.

Atkins and Stanbury (*4*) made a study of illumination and plant distribution in spruce, larch, oak, and holm oak woods. Under the shade of a dense spruce stand (*Picea excelsa*) light values were about 1 to 2 per cent, and only ivy and wood sorrel seemed able to survive. Increasing light up to about 9 per cent allowed Rubus, ash, hazelnut, and sycamore maple to come in. Larch and oak stands had about 10 to 15 per cent light and supported a good woodland flora. Holm oak reduced the light to 1 to 3 per cent. Under this only ivy seemed able to thrive.

Stallard (*14*) describes secondary successions in northern Minnesota forests. His paper contains a description of the undervegetation in Norway pine forests and the successional changes brought about by increasing shade. He gives curves showing the annual height growth of Norway, northern white, and jack pines from 1 to 28 years of age, growing in the shade and in the open. The curves for jack pine show rapid falling off in growth with shading, particularly under Norway pine canopies. Norway pine seedlings, on the other hand, appear able to grow at a reasonable rate when shaded by jack pine but not so rapidly as in the open. Northern white pine on heavy soils showed almost as rapid height growth in the shade of hazel, aspen, and birch (10 to 30 per cent light) as in the open. Stallard considers that Norway and white pines form the mature stage in the succession in coniferous forests of Minnesota.

THE STAND

This study was carried out on the Chippewa National Forest located in the north-central part of Minnesota. Along the shores of Cass Lake and Pike Bay are 10 sections of Norway pine forests which have been undisturbed by logging operations, except to remove dead and dying trees at 5-year intervals. All this area has been subject to fires at various times during the past. However, most of the fires have been comparatively light, since none of them killed many of the large trees. The trees average more than 200 years in age and are fine, tall specimens, typical of the virgin Norway pine stands of the Lake States. (Fig. 1.) The trees average about 90 feet in height and in many places stand as dense as their crowns will permit. Numerous openings up to 300 or more feet in diameter can be found in the forest. They were perhaps caused by fire, wind, or other destructive agencies. The undergrowth is composed mostly of woody shrubs, among which bearberry and blueberry are dominant. Many of the openings are growing up to vigorous stands of young Norway pine and others to hazelnut, oak, and paper birch. The lower vegetation throughout is fairly luxuriant and is indicative of the presence of ample moisture and light for satisfactory plant growth. Scattered through the Norway pines are occasional white pines, usually larger and overtopping the Norways. Under the old white pines there can almost invariably be found numerous white pine seedlings. Where Norway and white pine seedlings have an equal chance for establishment white pine seems to be more abundant. Jack pine seed trees

are less common than white pine and are usually found in groups in the larger openings or in more or less pure stands along the periphery of the old Norway pine. Where jack pine seed is available the seedlings are usually present and grow quite rapidly.

Wherever pine reproduction has become sufficiently well established to exclude most of the other vegetation it is entirely too dense for good growth. On practically all the plots examined the young pines were too crowded to make rapid growth or they were suffering from competition with other plants. Apparently not until the young saplings have attained a height of 10 to 20 feet is there sufficient expression of dominance for the leaders to make rapid growth. On only two of the plots included in this study had the stand reached this stage, and on each of them the dominant trees showed recent

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FIGURE 1.—Typical virgin Norway pine stand, with young Norway reproduction in the openings. These saplings are about 18 years old and 4 to 5 feet high

annual height increments of 1 foot and more. The average height growth for trees less than 6 feet high in crowded stands is about 0.3 foot.

The soil is of fluvioglacial origin. It is classed as fine sand and is weakly podsolized. The humus layer is usually less than 2 inches thick and tends to decompose rather rapidly. The gray leached layer is usually 6 to 8 inches thick, and the brown layer generally extends to a depth of 2 or more feet. During the dry period in the summer of 1930 practically all the available moisture was withdrawn to a depth of 2 feet. Below this depth the soil was in most places quite moist. Most of the plots examined lay less than 20 feet above the lake level.

A good crop of cones was produced by the old trees in the fall of 1930, and there is every reason to suppose that ample seed has been produced at intervals of from three to seven years during the past century. Absence of forest reproduction on any particular spot can, therefore, scarcely be attributed to lack of available seed in the past.

METHODS

All field work was carried out doing the summer of 1930. Fifty plots, approximately one-tenth acre in size, were laid out in groups of three to five and chosen to show differences in forest reproduction. A typical group would contain one plot without forest reproduction, one plot in which the forest reproduction was present but was making very poor growth, one plot in which the forest reproduction was making rapid growth, and perhaps one plot occupied by aggressive brush species. A temporary stake marked the center of each plot. The soil was examined to a depth of 8 inches. General notes were taken on the character of the stand, the date of the last fire, and the general character of the undergrowth.

Two quadrats, 0.001 acre each, were laid out at equal distances on opposite sides of the center stake. On these quadrats all coniferous reproduction was counted and the average age and height determined. The mean annual height growth of the conifers was found by dividing the average height by the average age.

All species of plants growing on each plot were listed and their relative abundance estimated in three classes: Class 1, less than one-twentieth of the area occupied; class 2, one-twentieth to one-fifth of area occupied; and class 3, more than one-fifth of area occupied.

LIGHT MEASUREMENTS

Measurements of total and diffuse radiation on cloudless days were made with a thermopile (*13*) at 10 points uniformly distributed over the area of each plot. The measurements were converted to percentages of the radiation intensities in the open and averaged for the entire plot. It was originally intended to use the readings of diffuse light as a basis for correlation, but it was found that a very considerable amount of direct sunlight is reflected from leaves, hence measurements of diffuse light included not only the sky light which penetrated the canopy but in addition a large amount of reflected sunlight. It was also found that measurements of diffuse light were only roughly correlated with measurements of total sunlight. Over a range of from 30 to 100 per cent of total sunlight, diffuse light changed from only 52 to 66 per cent.

It was felt, therefore, that the measurements of diffuse radiation made did not give an accurate evaluation of the light available to the undervegetation.

Measurements of total radiation, on the other hand, show such great variability that it is difficult to get a reliable estimate of the light available on a given area. The standard deviations for 10 readings ranged from 1 to 14 per cent.

TABLE 1.—*Standard deviations of light measurements by 10 per cent intensity classes*

Intensity class	Mean value	Plots	Standard deviation of mean	Intensity class	Mean value	Plots	Standard deviation of mean
	Per cent	*Number*			*Per cent*	*Number*	
0-9 per cent	4.0	5	1.1	50-59 per cent	56.0	8	4.3
10-19 per cent	17.0	1	4.0	60-69 per cent	63.5	6	4.9
20-29 per cent	25.0	9	2.8	70-79 per cent	75.0	2	8.7
30-39 per cent	36.0	5	4.6	80-89 per cent	89.7	1	9.0
40-49 per cent	44.0	5	5.1	90-100 per cent	92.0	2	1.0

The plots were grouped in 10 per cent light-intensity classes, depending upon the mean light values for each plot. Standard deviations were again calculated for each group of plots. These are given in Table 1. The points shown on the curves in Figures 2, 3, and 4 may therefore be considered to be in error as much as 5 per cent either way in light measurements. This is probably considerably less than the error in sampling the vegetation and coniferous reproduction.

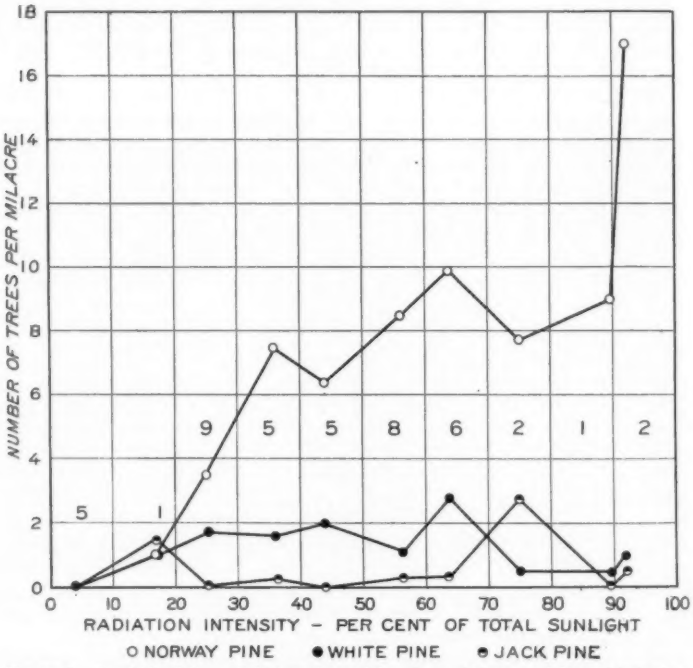

FIGURE 2.—Correlation between light intensity and abundance of Norway, white, and jack pines

ANALYSIS OF MATERIAL

Height measurements and number of trees per mil-acre (one-thousandth acre) were averaged for each plot. These were then plotted against light intensity and averaged by 10 per cent classes. (Figs. 2, 3, and 4.) Plots showing evidence of fire since 1918 were not included in determinations the results of which are shown in these figures. The number of trees per mil-acre in any class is obtained by averaging the number per mil-acre found on each plot in the class. The number of plots occurring in each class is shown by a numeral on the graph. The mean annual height growth is likewise averaged by light-intensity classes. In this case, however, plots having no coniferous reproduction were not used in finding the average point. The weight on a point shows the number of plots used in determining its position. The abundance of a plant in any 10 per cent light class is

arbitrarily represented by averaging its class numerals 1, 2, and 3 for all plots in their light class. Plots on which a given plant did not occur were not used in determining the abundance for the class. Frequencies were determined by dividing the number of plots on which a given plant occurred in an intensity class by the number of plots in the class. Abundance and frequency curves are shown in Figures 5, 6, and 7, with numerals showing the number of plots used

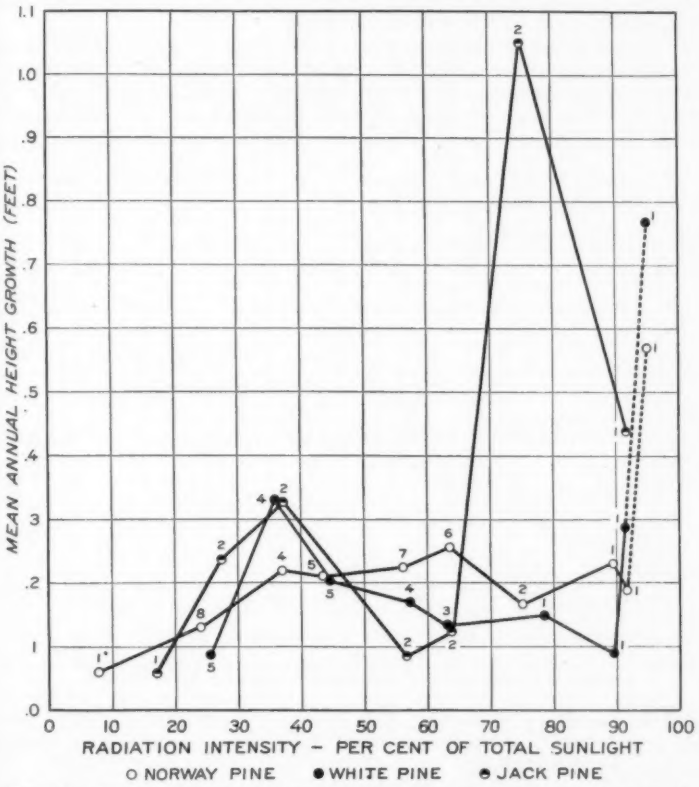

FIGURE 3.—Correlation between light intensity and the mean annual height growth of young Norway, white, and jack pines growing in a virgin Norway stand. Numerals indicate number of plots in each class

in determining a point. The weight on the abundance point divided by the weight on the corresponding frequency point gives the frequency. Only plants occurring on at least 10 plots are used in these figures.

LIGHT AND NATURAL REPRODUCTION

The number of trees per mil-acre is plotted against light in Figure 2. The poor showing of jack pine may be the result in part of insufficient light, but undoubtedly the chief reason for its sparsity is lack of seed trees. The comparatively small number of white pines established

is also due to lack of seed. The curve for Norway pine, on the other hand, well illustrates the correlation between light and establishment. None of the three species mentioned was able to become established where less than 5 per cent light was available. At 17 per cent light, establishment was fair—the equivalent of 3,000 trees per acre, which is about the minimum number for satisfactory natural reproduction. Any light value higher than 35 per cent seemed to be excellent for the establishment of Norway pine seedlings, as all plots averaged more than 6,000 trees per acre.

If Norway pine seedlings are not present on areas receiving 35 per cent light or more, no method of cutting the old stand is likely to better matters, since factors other than light are operative in keeping the pine out. The lowest average light intensity on any plot shaded only by old Norway pine was 17 per cent. This plot had the equivalent of

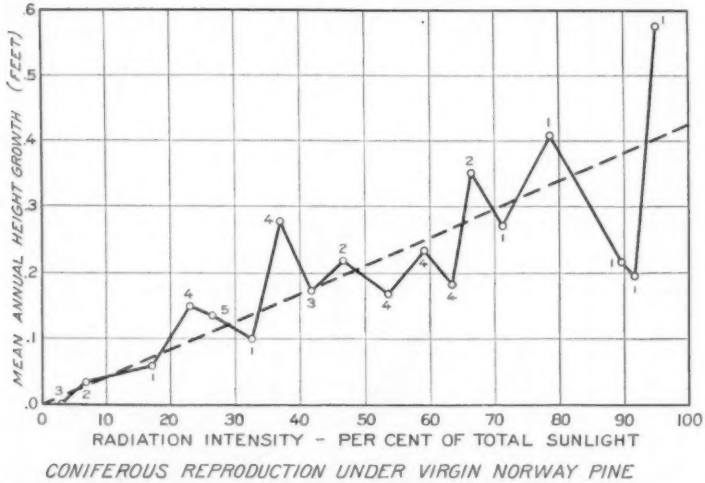

FIGURE 4.—Correlation between light intensity and mean annual height growth of stands of young Norway, white, and jack pines. Numerals indicate number of plots in each class

3,500 pine seedlings per acre. It seems, therefore, that virgin Norway pine stands are not likely to be so dense as completely to exclude coniferous reproduction.

Although the shade cast by the old Norway pines themselves may never be too dense for the establishment of reproduction, that cast by an understory of hazelnut, birch, alder, and other shrubs may quite effectively exclude pine seedlings. Fifty-five measurements taken of light beneath brush canopies gave values ranging from 0.7 to 15.8 per cent, with a mean of 4.4 per cent and a median of 3.5 per cent. Only rarely are pine seedlings able to grow through an understory of such density. In practically every case where the brush formed a continuous canopy, pine reproduction was either absent or in very poor condition. The whole study points to the importance of the undergrowth in determining coniferous establishment in virgin stands.

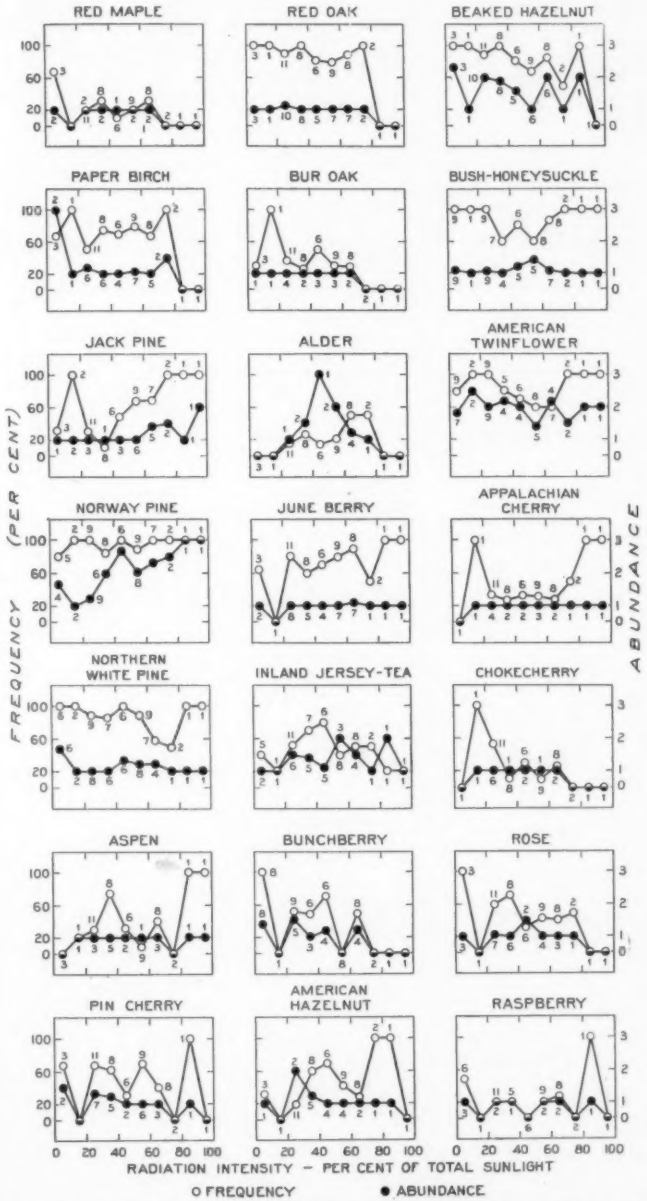

FIGURE 5.—Correlation between light intensity and the frequency and abundance of various woody plants found growing in a virgin Norway pine stand. Numerals indicate number of plots used to determine the point

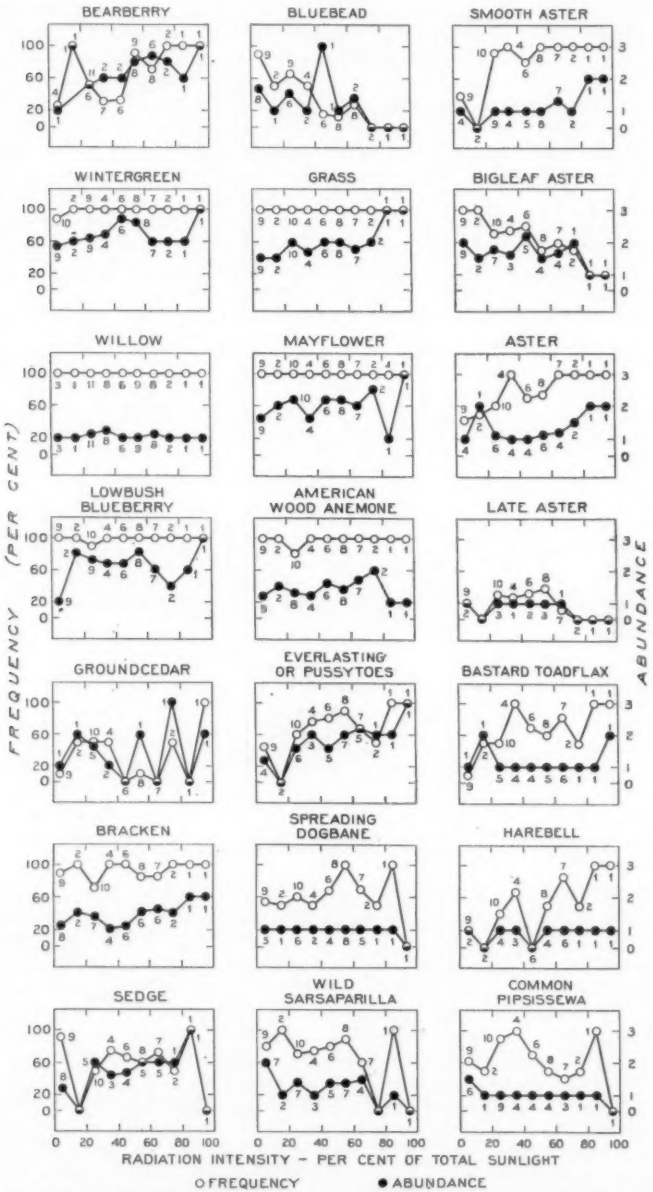

FIGURE 6.—Correlation between light intensity and the frequency and abundance of various woody and herbaceous plants growing in a virgin Norway pine stand. Numerals indicate number of plots used to determine the point

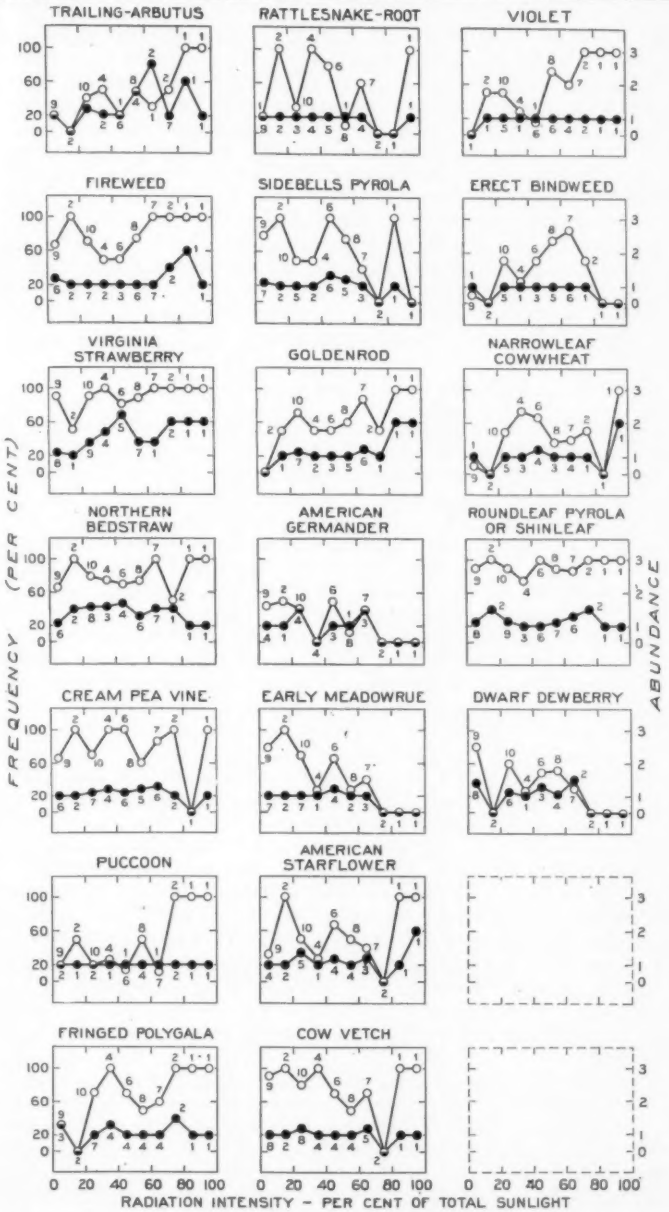

FIGURE 7.—Correlation between light intensity and the frequency and abundance of various herbaceous plants growing in a virgin Norway pine stand. Numerals indicate number of plots used to determine the point

The minimum light value in which Norway and white pines can exist seems to be between 1 and 5 per cent. Twenty-one measurements of light beneath dense Norway pine reproduction gave values ranging from 0.9 to 8.2 per cent with a mean of 3.4 per cent and a median of 3.0 per cent. No young seedlings were actually growing where these low values prevailed, yet the saplings themselves were able to reduce the light to that value. If it is assumed that green pine needles can not persist long in light values too low to supply energy for their own photosynthetic needs, then the minimum light requirements for survival would be approximately equal to the intensity prevailing beneath the very dense stands. This value is, however, probably nearer the minimum requirement for survival where other factors are favorable, and would be, therefore, much lower than the value required for establishment and growth of pines in the forest.

LIGHT AND HEIGHT GROWTH

In Figure 3 the mean annual height growth for Norway, white, and jack pines is plotted with light intensity as abscissas. Both white pine and jack pine were usually sparse in the understory; hence the curves show great irregularities. Norway pine, on the other hand, shows a fairly regular trend. It can be seen that up to about 63 per cent light, at which point Norway pine showed a maximum, the growth increases fairly regularly with increasing light. Jack pine shows a maximum at 75 per cent and white pine at 36 per cent. The ability of a species to attain maximum height growth at low light values may be considered as a measure of its relative tolerance. The relative tolerance of the three species, as determined by this method, is, in decreasing order, white pine, Norway pine, and jack pine. This is the order of tolerance commonly accepted in this region.

At this point it is well to consider the actual maximum values. White pine showed a maximum mean annual growth of 0.33 foot or 4 inches; Norway pine of 0.25 foot or 3 inches; and jack pine of 1.05 feet or 12½ inches. Except for jack pine, the maxima are low. They do not represent the maximum possibilities of the species, but rather the average maximum growth attained in crowded stands beneath an upper canopy. One plot, located in the center of an opening about 200 feet in diameter, is covered with a dense stand of Norway and white pines approximately 26 years old and 20 to 30 feet in height. White pine has grown more rapidly than Norway pine and apparently will soon almost completely occupy the area. The true maximal height growth, therefore, for Norway and white pines is much greater than 3 to 4 inches a year and seems to occur in full sunlight rather than in shade. This is indicated by the last point on the curves of Figure 3.

In Figure 4, the mean annual height growth of Norway, white, and jack pines is weighted for the actual number of trees of each species on each mil-acre quadrat. This gives a better picture of the actual growth of the stands of young reproduction, since the species which had gained ascendancy on a given plot seemed to be related to chance as well as to light values. A straight line is drawn on the chart to show how nearly the growth is proportional to the light intensity.

Approximately 35 per cent light appears to be necessary for reasonably good growth. In light values below 20 per cent growth is too

slow to insure thrifty development of the young stand. In fact, trees showing only one-half inch yearly growth are generally extremely unhealthy or actually dying.

LIGHT AND THE GROUND COVER

The frequency and abundance of other plants are plotted against light intensity in Figures 5, 6, and 7. The curves given are based on estimates rather than accurate counts; hence only general trends are considered significant. In studying these curves, particularly the frequency, attention must be paid to the weights of the points, as any point with a weight of 1 can have only frequencies of 0 or 100 per cent and one with a weight of 2 may have in addition only 50 per cent.

TABLE 2.—*Classification of ground cover as to abundance under different light intensities*

Cover never abundant	Cover abundant at high intensities	Cover abundant at intermediate intensities	Cover abundant at low intensities
Paper birch.	Jack pine.	White pine.	Bunchberry.
Red maple.	Norway pine.	Alder.	American twinflower.
Bur oak.	Inland Jersey-tea.	American hazelnut.	Groundcedar.
Red oak.	Bearberry.	Beaked hazelnut.	Bluebead.
Aspen.	Bracken.	Dwarf bush-honeysuckle.	American germander.
Thicket shadblow or June berry.	Sedge.	Rose.	
Pin cherry.	Grass.	Wintergreen.	
Appalachian cherry.	Mayflower.	Lowbush blueberry.	
Chokecherry.	American wood anemone.	Wild sarsaparilla.	
Willow.	Pusstyoes or everlasting.	Bigleaf aster.	
Red raspberry.	Aster.	Trailing-arbutus.	
Spreading dogbane.	Smooth aster.	Northern bedstraw.	
Late aster.	Virginia strawberry.	Dwarf dewberry.	
Harebell.	Goldenrod.		
Common pipsissewa.	Fireweed.		
Cream pea vine.			
Puccoon.			
Fringed polygala.			
Rattlesnake-root.			
Roundleaf pyrola or shinleaf.			
Sidebells pyrola.			
Early meadowrue.			
Cow vetch.			
Violet.			
Erect bindweed.			
Bastard toadflax.			
American starflower.			
Narrowleaf cowwheat.			

TABLE 3.—*Classification of ground cover by tendency to high frequency of occurrence under different light*

Indefinite	Greatest at high intensities	Greatest at intermediate intensities	Greatest at low intensities
Pin cherry.	Paper birch.	Red maple.	White pine.
Red oak.	Jack pine.	Aspen.	Bur oak.
Dwarf bush-honeysuckle.	Norway pine.	Inland Jersey-tea.	Bunchberry.
Wintergreen.	Alder.	Rose.	Beaked hazelnut.
Appalachian cherry.	Thicket shadblow or June berry.	Spreading dogbane.	American twinflower.
Red raspberry.	Bearberry.	Late aster.	Chokecherry.
Willow.	American hazelnut.	Common pipsissewa.	Groundcedar.
Lowbush blueberry.	Sedge.	Bastard toadflax.	Bluebead.
Grass.	Pussytoes or everlasting.	Erect bindweed.	Wild sarsaparilla.
Mayflower.	Smooth aster.	Narrowleaf cowwheat.	Bigleaf aster.
Bracken.	Aster.	Fringed polygale.	Northern bedstraw.
American wood anemone.	Harebell.	Rattlesnake-root.	American germander.
Cream pea vine.	Trailing-arbutus.	Sidebells pyrola.	Early meadowrue.
Puccoon.	Fireweed.	American starflower.	Cow vetch.
Roundleaf pyrola or shinleaf.	Virginia strawberry.		Dwarf dewberry.
	Goldenrod.		
	Violet.		

An examination of the curves in Figures 5, 6, and 7 shows that several species exhibit the same general tendencies in their response to light. In Table 2 they are separated into four groups—those never abundant, those most abundant at high light intensities or tending to become more abundant with increasing light, those most abundant at intermediate light intensities, and those most abundant at low light intensities. This table shows also that the number of species in each group decreases with decreasing light intensities.

The frequency of plants in a given light intensity class is perhaps more subject to chance variation than the abundance; hence, the frequency curves show more irregularities. Some curves are too irregular to show any definite trend. In Table 3 the plants are grouped according to their tendencies to be most frequent at high, intermediate, or low light values.

Examination of Tables 2 and 3 will show that certain species appear to be misplaced. Aspen, beaked hazelnut, and bur oak are ordinarily considered intolerant and are usually found growing best on moderately heavy soils. When these plants occur in Norway pine forests they appear to do better in the shade. This condition would probably be reversed on a better soil.

COMPETITORS OF CONIFERS

The most serious competitors of young conifers starting under the old Norway pine stand are beaked and American hazelnut, inland Jersey-tea, bracken, grass, sedge, lowbush blueberry, and bearberry. Of these, hazelnut and lowbush blueberry seem to thrive best in light shade, but the others increase in abundance and in vigor with increasing light. As Norway pine does very poorly in less than 35 per cent light, it would seem best to maintain intensities of about 35 to 50 per cent light (which corresponds to a crown density of about one-half to two-thirds in virgin stands) until the Norway pine is sufficiently well started to be out of danger from competition by grass, sedge, bearberry, and blueberry, then to increase the light value by degrees up to 100 per cent. Of course, regulation of crown density alone will not free Norway pine from competing vegetation, but, by maintaining a crown density which is more favorable for Norway pine than for some of its active competitors, the pine should have a better chance to gain ascendency.

RELATION OF LIGHT STUDIES TO SILVICULTURE

The results of this study indicate that both the establishment and growth of young pines in a virgin Norway pine stand are definitely correlated with light intensity. Individual plots show considerable deviation from the general average, but these deviations are due in large part to inaccuracies in sampling the light intensity, inaccuracies in sampling the coniferous reproduction, and certain extraneous factors, chief of which is the competition of other vegetation. The study also indicates that light as a growth factor operates in the same way in the forest as in carefully controlled experiments with artificial conditions. The full effect of added light is, however, not always utilized in the forest because other growth factors may not be favorable.

Virgin Norway pine stands 200 years old or older are not likely to be so dense that Norway seedlings can not grow underneath the old trees. If, on the other hand, alder, hazelnut, birch, and other plants become established in the openings in a virgin stand, they may quite effectively exclude all conifers. Removing the remaining old trees from over the underbrush is not likely to result in conifers becoming established unless the brush cover is broken up by logging operations. In marking trees for cutting at the end of a forest rotation, attention should be paid to the undergrowth as well as the mature trees, as a stand of shrubs and aggressive herbs may rapidly take over the area before conifers can be established. On areas devoid of reproduction and having 35 per cent or more light, some other treatment than cutting the old stand should be given to bring in the desired stand of young trees.

The gradual enlargement of small openings in which reproduction is already established would seem to be the ideal method not only for getting a good establishment of reproduction in an old stand but also for providing best conditions for its subsequent survival and growth. Such a method, which is essentially the group selection system, also tends to discourage the active competitors of conifers which seem no more able to thrive beneath a dense canopy of young pine than the pine is able to thrive beneath dense underbrush.

These recommendations as to proper silvicultural treatment are at present offered chiefly as suggestions, since their application to areas outside the stand actually investigated has not been demonstrated.

SUMMARY AND CONCLUSIONS

In a virgin Norway pine stand studied in relation to light and to other vegetation, approximately 35 per cent light, or a crown density of about two-thirds, seems to offer satisfactory conditions for the establishment of Norway pine seedlings. Light values below 17 per cent result in uncertain establishment. The number of trees per acre seems to continue to increase with the light up to the intensity of full daylight.

The height growth of Norway pine increased with increasing light up to 63 per cent. White pine showed a maximum growth at about 36 per cent light, and jack pine at 75 per cent. These maxima are believed to be apparent maxima applying only to the conditions in the stand studied. The fact that white pine attains maximum height at lower values than Norway pine, and Norway pine at lower values than jack pine indicates that white pine is the most tolerant of shade and jack pine least. This is in agreement with current opinions based upon other methods of study.

When the growth of the stand is considered instead of the growth of individual species, there is a more definite correlation between the light intensity and height growth, which tends to approach a linear relationship.

The light intensities commonly prevailing in virgin stands of Norway pine are not likely to be too low for the establishment of reproduction. On the other hand, understories of hazelnut and other shrubs, which reduce the light to less than 5 per cent, quite effectively exclude coniferous seedlings.

In virgin Norway pine forests, the presence of an understory of shrubs is just as important in determining the establishment of pine seedlings as the density of the old stand.

The group selection cutting system most nearly duplicates the conditions in a virgin stand which appear most satisfactory for the establishment and growth of the young trees.

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GROWTH RECORD OF FERTILIZED APPLE TREES GROWN IN METAL CYLINDERS[1]

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INTRODUCTION

In 1908 the Pennsylvania Agricultural Experiment Station began a series of fertilizer experiments in commercial apple orchards in several sections of the State. The results (*7*, *8*, *9*)[2] were so variable that in many of the orchards it was difficult to find significant differences between contrasted treatments. Studies of these orchards and of the resulting data indicated that four factors were chiefly responsible for the variability of the results: Initial differences in soil fertility; differences in methods of management, especially the use of sod as compared with tillage; slope, which not only created initial fertility differences, but continued to accentuate them during the continuation of the experiment; and genetic differences affecting the vigor of the seedling roots.

METHODS OF EXPERIMENTATION

In 1918 it was decided to begin at State College a fertilizer experiment in which as many as possible of these variables would be eliminated or considerably reduced in effect through the use of metal cylinders to confine the roots in uniform soil. At the time this experiment was being planned, the Delaware Agricultural Experiment Station was growing peach trees in large concrete pits and the Florida station (*3*) had citrus trees growing in eight galvanized-iron tanks, 5 feet 3 inches by 4 feet; but, as far as the authors know, no successful attempt had been made to grow apple trees to maturity in this manner. While this experiment was under way, a number of experimenters successfully used dwarf apple trees in pots in fertilizer studies. The recent work of Davis is an example (*4*).

To secure greater uniformity of root growth it seemed desirable to use vegetatively propagated roots of a single clon. Through the cooperation of R. G. Hatton, of the East Malling Research Station, Kent, England, the requisite number of Malling Type 12 apple roots were forwarded in January, 1920. Unfortunately, this shipment was lost in transit. The following year Professor Hatton sent a second shipment of the same stock, but was obliged to include stock of two ages—some that had been removed from the mound layer and grown one year in the nursery row and some that had just been separated from the mounds.

These roots were planted in a nursery at State College in the spring of 1921. Ten days later the older stocks were cut off and whip grafted with scions from a 10-year-old Stayman Winesap tree in the

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[2] Reference is made by number (italic) to Literature Cited, p. 265.

college orchard, which, in turn, had been propagated from a tree used in one of the fertilizer experiments begun in 1908. In the summer of 1921 the remaining stocks were budded from the same tree.

In the meantime, 42 cylinders, or "rims," of ⅜-inch boiler plate 5 feet across and 5½ feet deep had been secured. These were sunk in the ground to within 6 inches of the top at the corners of 20-foot squares, making 6 rows of 7 cylinders each. The area of each rim was approximately one twenty-two-hundredths of an acre. Each rim held about 5 tons of soil. The bottoms of the cylinders were not closed, but were set on several inches of coarse crushed limestone. The first 3 or 4 inches of the filling were of this material. It was expected that this layer of stone would prevent the downward movement of the tree roots, but this proved not to be the case.

The soil used to fill these cylinders was taken from an area on the college farm that, as far as could be determined, had received little or no fertilizer. The soil was dug in three layers, the first, 9½ inches deep; the second, 9½ to 18½ inches; and the third, from 18½ to 60 inches. Each layer was thoroughly mixed and placed in the rims in its original order. Water was run in when the soil was placed in the rims to assist in compacting it. This work extended through the fall of 1919 and the spring of 1920.

The soil used is classified as a Hagerstown silty clay loam. It has been formed in place by the weathering of the limestone of the lower Silurian formations. As the rims were filled, samples were taken for chemical and mineralogical analyses. The results of these examinations have been presented by Thomas (*10*). The soil is rich in a wide range of the rarer elements. Phosphorus is low, the analysis showing only about 0.1 per cent P_2O_5, while potassium is high, with K_2O averaging over 4 per cent.

Because of the loss of the first shipment of stocks, trees were not available for planting in the rims until the spring of 1922. In the meantime, crops were grown in the rims and worked into the soil to improve its fertility.

PLANTING AND EARLY CARE OF TREES

The first planting was made in May, 1922. The four inner rows of seven rims each were filled with the older stocks which had been whip grafted the previous spring. On April 19, 1923, the two outer rows were planted with the stocks that had been budded. Table 1 shows the weights of these trees at planting and their location.

TABLE 1.—*Weights of trees at time of planting*

Row	Weight (grams) of trees in row indicated					
	F [a]	E	D	C	B	A [a]
No. 1	375	130	130	120	135	385
No. 2	370	140	115	115	135	350
No. 3	350	150	110	110	175	345
No. 4	340	180	100	100	180	335
No. 5	325	180	90	[b] 430	195	315
No. 6	315	210	85	85	195	310
No. 7	290	215	75	75	230	902

[a] Rows A and F were planted in 1923, 1 year later than the other rows. The later-planted trees were larger at the time of planting.

[b] This tree was also planted in 1923. It replaced the original tree which had been badly injured with a cultivating tool.

Because of the limited number of stocks available, it was not possible to make a close selection of the planting material. However, the smaller number needed when the two outer rows were planted made it possible to select fairly uniform material for these rows. The coefficient of variability with respect to weight of trees for the 28 in the middle four rows was 31.9 ± 3.2; for the outer two rows, 8.5 ± 1.1.

The fertilizer treatments were planned to run north and south, with six trees in the row and seven rows available for contrasting treatments. (Fig. 1.) Table 1 shows the attempt that was made to smooth off the variability of the material at the first planting. The two lightest trees were planted in the two central rims of the east row and the two heaviest in the two adjoining rims of the same row. There is a progressive increase from east to west in the tree weights in the central two rows of trees, and from west to east in the adjoining two rows. Thus there is a high degree of variability among the trees in the east row but four quite uniform trees in the west row.

The entire area received the same treatment until May, 1924, when the trees had become well established. Each year the trees were hoed during the spring and summer, and winter cover crops were grown.

Early in the planning of this experiment it was seen that many of the problems could be solved only by chemical studies. William Frear, chemist of the Pennsylvania Experiment Station, made a preliminary study of available methods of attack, with the assistance of Walter Thomas, of the Department of Agricultural and Biological Chemistry. Since the death of the former in 1922, Doctor Thomas has had charge of the chemical research connected with this project, while the Department of Horticulture has had charge of the care of the trees and the taking of all field records.

The only pruning given the trees while they were under uniform treatment was to remove the wood needed for chemical analyses, most of the material being taken from the trees that had made the heaviest growth. The correlation between the total branch elongation of the trees for 1922, 1923, and 1924 and the length of the prunings removed from the trees was 0.37 ± 0.09. This heavier pruning of the more vigorous trees helped to increase the uniformity of the block.

As long as this experiment was in progress, trunk circumference and branch elongation were measured each year, but not spur growth. All growths under 5 cm. were considered potential spurs. In 1922 the leaves were secured at leaf fall and weighed. In after years leaf samples were weighed, but no estimates of the total leaf area or weight were made until 1927 at the time the trees were dug up.

The coefficient of variability of the first year's total branch elongation for the 42 trees was 46.3 ± 4.1. This included the 1922 growth of rows B through E and the 1923 growth of rows A and F. When these 1923 records of rows A and F were used with the same year's records for the other rows, the coefficient of variability for total branch elongation was 24.2 ± 1.9, and for trunk diameter, 12.9 ± 0.97. These figures indicate that the outer rows, though planted a year later, are fairly comparable to the inner four rows because of their larger size at planting.

A mixture of bluegrass and timothy was seeded in half of the rims on May 27, 1924. The coefficient of variability of the branch elonga-

tion for the trees in sod during 1924 was 17.5 ± 1.88; for those under cultivation, 27.1 ± 3.02. The coefficient of variability for trunk diameter of all the trees was 14.3 ± 1.07.

From 1920 through 1923, two to three green-manure crops were grown annually in the rims. Whenever possible, records of height or weight of these covers were taken. These records were reduced to percentages, the rim with the heaviest cover crop each time being rated at 100. Table 2 shows the average percentages of the five cover crops which were measured, each being recorded in percentage of the largest cover of the respective crop. As judged by the growth of these cover crops, the soil in the rims showed a high degree of uniformity.

TABLE 2.—*Average of five cover crops before fertilization, expressed in percentage of the heaviest cover of each cover crop*

Row	Percentage crop cover in row indicated						Average
	F	E	D	C	B	A	
No. 1	73	60	60	58	52	74	63
No. 2	81	67	71	69	58	76	70
No. 3	66	55	62	58	67	90	66
No. 4	77	65	70	64	58	78	69
No. 5	81	66	72	79	51	78	71
No. 6	79	66	68	75	70	62	70
No. 7	66	57	61	73	63	73	66
Average	75	62	66	68	60	76	

FERTILIZER TREATMENTS

Figure 1 shows a diagram of this block with the fertilizer treatments indicated. Table 3 gives the weights of the various salts used and the time of application. All fertilizers were chemically pure materials. It was originally intended to use amounts equivalent to 50 pounds of N, 100 pounds of P_2O_5, and 50 pounds of K_2O per acre for trees standing 100 to the acre. The reasons for changes from this plan will be mentioned later.

TABLE 3.—*Time of fertilizer applications and quantities used for each rim*

Treatment and date applied	Weight (grams) and kind of fertilizer used in row indicated						
	1 (P)	2 (N)	3 (N,P,K)	4 (check)	5 (P, K)	6 (N,K)	7 (N,P)
$NaNO_3$:							
Apr. 18, 1925		906	906			906	906
May 3, 1926		45	45			45	45
June 7, 1926		453	453			453	453
June, 1926		408	408			408	408
May 5, 1927		337	337			337	337
May 18, 1927		338	338			338	338
June 10, 1927		337	337			337	337
$CaH_4(PO_4)_2H_2O$:							
Apr. 18, 1925	534		534		534		534
May 3, 1926	267		267		267		267
June 7, 1926	267		267		267		267
May 5, 1927	534		534		534		534
May 18, 1927	267		267		267		267
K_2SO_4:							
Apr. 18, 1925			293		293	293	
May 3, 1926			147		147	147	
June 7, 1926			147		147	147	
May 5, 1927			293		293	293	
May 18, 1927			147		147	147	

Nitrogen and phosphorus were employed as single element treatments but potassium was not. The Hagerstown soil used in this experiment is well supplied with potassium, and none of the orchard tests previously conducted in this State had shown any clear evidence of a potash response.

Numerous field experiments have indicated that the nature of the fertilizer response is very materially altered by the presence or absence of grass in the orchard. Because of this fact, the south half of each row of rims (trees D, E, and F) was seeded to bluegrass on May 27, 1924.

The first fertilizer treatment was not made until the spring of 1925; hence there was nearly a year during which half of the block was under an unfertilized sod. It was not expected that this new sod would materially influence the growth of the trees in such a relatively short time, but Table 4 shows that it did to a significant extent in the majority of the rows.

The low odds in row 5 were due to the fact that one of the cultivated trees had been replanted. In row 7 one of the largest trees growing in sod had been killed back to within a few inches of the bud during the winter of 1923–24, and consequently made a heavy renewal growth in 1924. Table 4 shows that the trees growing in sod started the period of fertilization with a slight handicap.

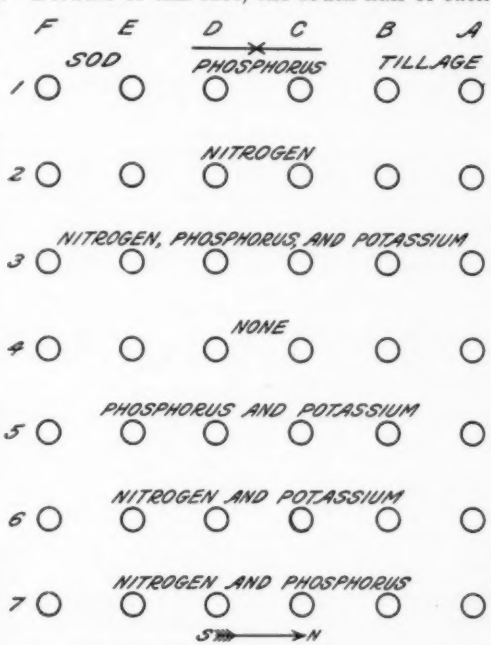

FIGURE 1.—Diagram indicating rims, or cylinders, containing the trees, which were located at the corners of 20-foot squares; the fertilizer treatments used are also shown

The first application of fertilizer was only two-thirds of the amount intended to be used as an annual application. In spite of this reduction, the concentration of these amounts in the small area of a single rim resulted in serious burning of the grass. For this reason the other third was not put on in 1925, and in later years the amounts used in a single application were further decreased. Even then there was, at times, some burning of the grass.

The injury to the grass from the fertilizer was usually greatest where nitrate of soda was used, but even in rims which received only phosphorus some injury was evident. In nearly all cases the injury was temporary, and the grass was quickly replaced by a new and vigorous growth. On any portions where the grass roots were killed

the ground was sodded over with grass grown in a bed which had been filled with the same soil as the top layer in the rims.

TABLE 4.—*Comparative branch elongation of trees in cultivated and in sodded rims, 1924*

Row No.	Treatment	Odds [a] in favor of cultivated trees	Row No.	Treatment	Odds [a] in favor of cultivated trees
1	P	11 to 1.	5	P, K	3 to 1.
2	N	102 to 1.	6	N, K	102 to 1.
3	N, P, K	226 to 1.	7	N, P	7 to 1.
4	None	102 to 1.			

[a] The significance of the difference between any two treatments has been estimated by the use of Student's method for interpreting paired experiments (*6*). Where, as in this table, the comparison is between sod and tillage, the pairing has been A–F, B–E, C–D. (Fig. 1.) When contrasting fertilizer treatments have been compared, the pairs have been those with similar letters.

Statisticians are agreed that odds of 30 to 1 may be accepted as clear indication that the difference is significant. Significant odds simply mean that the contrasted data differ by an amount too large to be due to chance. One is justified in placing reliance on the interpretation of this significant difference only in proportion to the success in eliminating all other variables except the treatment under study—a condition very difficult to comply with in horticultural research.

Whenever the grass was high enough, it was cut with hand shears and the clippings weighed. Table 5 gives the weights of these cuttings. There were large differences in sod growth under the different treatments.

TABLE 5.—*Total weights per rim of grass clippings made during 1925, 1926, and 1927*

Row	Treatment	Weight (grams) of grass clipped on lettered rim indicated			Average
		F	E	D	
No. 1	P	1,160	850	787	932
No. 2	N	2,860	2,240	3,963	3,021
No. 3	N, P, K	4,870	4,920	3,058	4,283
No. 4	None	670	490	445	535
No. 5	P, K	1,490	750	1,030	1,090
No. 6	N, K	1,970	2,530	1,733	2,078
No. 7	N, P	4,260	5,600	4,215	4,692

The burning of the grass through the use of nitrate of soda has already been described. The application of the same amount of fertilizer to the rims under cultivation modified the clay and affected the physical condition of the soil, making it less friable. This change in condition was sufficiently pronounced to be noted by field men who hoed the rims. The addition of the other fertilizer materials to the nitrogen fertilizer did not modify this effect. The rims under cultivation that did not receive nitrogen remained in excellent condition throughout the test. The addition of the chopped rye described below probably helped to improve the soil structure.

COVER CROPS AND WATERING

The oldest field fertilizer experiments in the United States are located on the college farm on soil very similar to that used in the rims. At the end of 40 years Gardner et al. (*5*, *p.* [*3*]), stated: "Phosphoric acid is the first limiting factor in this soil and until this element is supplied, nitrogen and potash give very little increase in yields."

The first application of fertilizers in the rims was made early in 1925. At that time there was a rye cover crop in the cultivated rims. Partly because of the fertilization, and partly from unknown causes, the growth of the rye cover crop in the different rims varied considerably when the crop was ready to be spaded under in late May. To meet this contingency, several beds had been built and filled 1 foot deep with soil similar to the topsoil in the rims. These were sown to rye when the rims were seeded. The rye in each rim was cut and the tops weighed. Enough rye in one of the beds was then cut to make the total weight of the tops in rim and bed 1 kg. The roots of this area in the bed were then dug and all tops and roots spaded into the rim. Thus each rim under cultivation received 1 kg of tops and the roots which went with those tops. No attempt was made to balance the organic matter in the sod rims.

Because of the variations in growth of the cover crops no more were seeded in the rims receiving cultivation. In the fall of 1925, rye was again sown in the beds. On May 20, 1926, this rye was dug, the dirt shaken from the roots, and the tops and roots chopped up together. Two kilograms of this chopped rye were added to each rim and spaded into the soil. The same method was used in 1927. In this way organic matter was kept from being a serious variable in the cultivated rims.

Early in the experiment it was evident that the normal moisture of the restricted soil of the rims would not be sufficient to maintain tree growth. Whenever the soil became too dry as indicated by soil-moisture determinations, from 1 to 2 inches of water were added to each rim. All trees under cultivation received the same application, and all trees growing in sod were treated alike, but the latter received more frequent applications. If there was excess water at any time, it would drain away freely through the crushed limestone under the rims into the well-drained soil below.

These applications of water did not maintain a sufficiently uniform moisture supply to meet the needs of the trees at all times. During a very dry period in the middle of July, 1927, the soil moisture dropped close to the point of physiological wilting. The drought was followed by a period of wet weather. Between 50 and 60 per cent of the apples on all trees cracked during the wet weather. This cracking is characteristic of the Stayman apple under irregular moisture supply. A few fruits cracked after a dry period in August of the same year. If this irregularity in moisture supply influenced the final results, it should have produced the greatest effect on the most vigorous trees. However, at no time was there any indication of wilting of the leaves, nor was there any indication that normal branch elongation was checked.

ESCAPE OF ROOTS FROM THE RIMS

The inner four trees in each row were planted in 1922 and the end trees a year later. During 1926 the older trees, with three exceptions, did not make as much total branch elongation as the younger trees, either under sod or in tillage, regardless of fertilizer treatment. It is probable that the restricted volume of soil was slowing up the growth of the older trees, but was still sufficient for the younger trees.

One of the older trees, D–3, growing in sod and receiving the full fertilizer treatment, was considerably more vigorous than any of the

other trees receiving either this combination or nitrogen and phosphorus, the combinations that gave greatest growth. During the summer of 1927 this tree was again abnormally vigorous, and the growth of two or three others also seemed longer and the leaves greener than on other trees under similar treatments. Because of these variations, it was decided to explore outside the rims to find whether any roots had penetrated the crushed stone in the bottom of the rims and reached the outer soil. Pits were dug in the late summer of 1927, and about half of the bottom edge of rim D–3 was exposed. One root over 2 feet long, probably in its second season of growth, was found, another about 18 inches long, and several small ones. From about one-third of the circumference of rim A–3, in which the tree was a year younger, 25 g of roots were secured. The longest was not more than a foot and a half, and evidently all were of that season's growth. Other rims in each treatment were partly uncovered; in each case some roots, ranging from small fibers to roots several inches long, were found extending into the surrounding soil. This discovery led to the decision to dig out all the trees in the fall of 1927 and to terminate this phase of the experiment.

Did the escape of roots influence the results enough to destroy their value? When all the trees were taken out of the rims in the fall of 1927, studies were made of root distribution, and any roots penetrating beyond the bottom of the rims were noted. Roots, other than a few small fibers, were found outside of only 17 of the rims. In 4 of these rims (B–1, B–2, D–3, F–1) the roots appeared to be in their second season of growth. If the performance of these four trees is judged by their total branch growth during 1927, all seem to show somewhat greater growth than the performance of similar trees would lead one to expect. While the presence of the younger roots outside the rims probably influenced the trees which produced them, there is no consistent evidence that this influence was great enough materially to change the nature of the results.

Two of the four trees with older roots outside the rims (B–2, D–3) received nitrogen. Any added vigor due to the escape of these roots would serve to accentuate the value of nitrogen in the 1927 records. The importance of this element in the experiment had been established with considerable certainty before this time; consequently, while the extent of some of the differences may have been influenced by the escape of the roots, the relative nature of the records would not be materially affected.

LEAF WEIGHT AND BRANCH ELONGATION

When the trees were dug out in the fall of 1927, the following records were taken: Weight of fruit, weight of leaves, 1927 branch growth, total weight of tops, diameter of trunks, and total weight of roots. The weights were all of fresh materials. Some samples were preserved for chemical analysis, and dry weights of these were determined. Since two papers (*12*, *11*) have been published in which certain of the chemical analyses made during the progress of this experiment have been reported, this phase of the work is omitted from the present paper.

From September 21 to 30, 1927, all the leaves were picked from the trees and weighed. Table 6 gives the fresh weights.

All of the highest 12 trees with respect to leaf weight received nitrogen, either alone or in combination; half of these were in rims receiving cultivation. Experiments in the college orchards since the planting of the trees, in 1908, which are similar in nature to these more carefully controlled tests, failed to show (*1*, *2*) any clear response to nitrogen applications from trees under cultivation until the orchard was at least 15 years old. The very positive nitrogen return in leaf weight during the third season of the fertilizer applications in the case of the rim trees under cultivation shows how much the use of the confining rim hastened root crowding and soil exhaustion. These differences are shown clearly in Figures 2, 3, and 4. Among the trees receiving nitrogen, those under sod had practically as heavy a leaf crop in 1927 as did those under tillage.

TABLE 6.—*Weights of green leaves picked from apple trees, September, 1927*

Row	Treatment	Weight (grams) of leaves from trees in sod				Weight (grams) of leaves from trees in tillage			
		F	E	D	Average	C	B	A	Average
No. 1	P	1,830	998	1,295	1,374	2,169	3,145	2,435	2,583
No. 2	N	3,460	2,314	2,800	2,858	2,113	4,415	4,060	3,529
No. 3	N, P, K	2,620	3,791	4,975	3,795	3,623	3,440	4,690	3,918
No. 4	None	529	1,192	1,220	980	1,882	1,855	1,950	1,896
No. 5	P, K	1,450	950	1,115	1,172	2,140	1,493	2,600	2,078
No. 6	N, K	3,240	2,397	3,370	3,002	2,795	2,600	2,600	2,665
No. 7	N, P	3,810	3,880	4,256	3,982	2,998	4,260	3,630	3,629

Trees receiving phosphorus showed a definite increase in leaf weights in 1927 over trees similarly treated but not receiving that element. (Fig. 2 compared to fig. 3.) There were three treatments of three trees each, both under sod and under tillage, in which phosphorus was checked against no phosphorus (N, P, K, against N, K; N, P, against N, and P against nothing). In the comparison of these treatments by Student's method, the odds that the trees receiving phosphorus had significantly greater leaf weights than those not receiving phosphorus, under sod and under tillage separately, were both about 68 to 1.

Not one of the 12 trees highest in leaf weight in 1927 stood in the row receiving nitrogen and potassium; in fact, the highest ranking tree in this treatment was fourteenth. The odds by Student's method that the trees receiving the N, K treatment had significantly less leaf weight than those in the adjoining N, P treatment are 276 to 1; (fig. 3, C6 and D6, compared to fig. 4) while the odds that the addition of K to N alone increased the leaf weights are only 3.5 to 1.

TABLE 7.—*Total branch elongation of apple trees during 1925, 1926, and 1927*

Row	Treatment	Length of branches (centimeters) in sod rims indicated				Length of branches (centimeters) in tillage rims indicated			
		F	E	D	Average	C	B	A	Average
No. 1	P	6,249	2,022	2,429	3,567	6,142	9,762	6,677	7,527
No. 2	N	10,764	5,070	8,321	8,052	4,679	12,003	11,759	9,480
No. 3	N, P, K	8,132	12,116	18,883	13,044	11,363	11,822	17,503	13,563
No. 4	None	2,203	1,780	1,740	1,908	4,080	4,207	6,166	4,818
No. 5	P, K	4,518	1,786	2,369	2,891	7,201	3,192	10,012	6,802
No. 6	N, K	8,386	5,660	9,212	7,753	6,738	7,411	7,900	7,350
No. 7	N, P	15,075	13,995	13,162	14,077	8,568	9,609	11,500	9,892

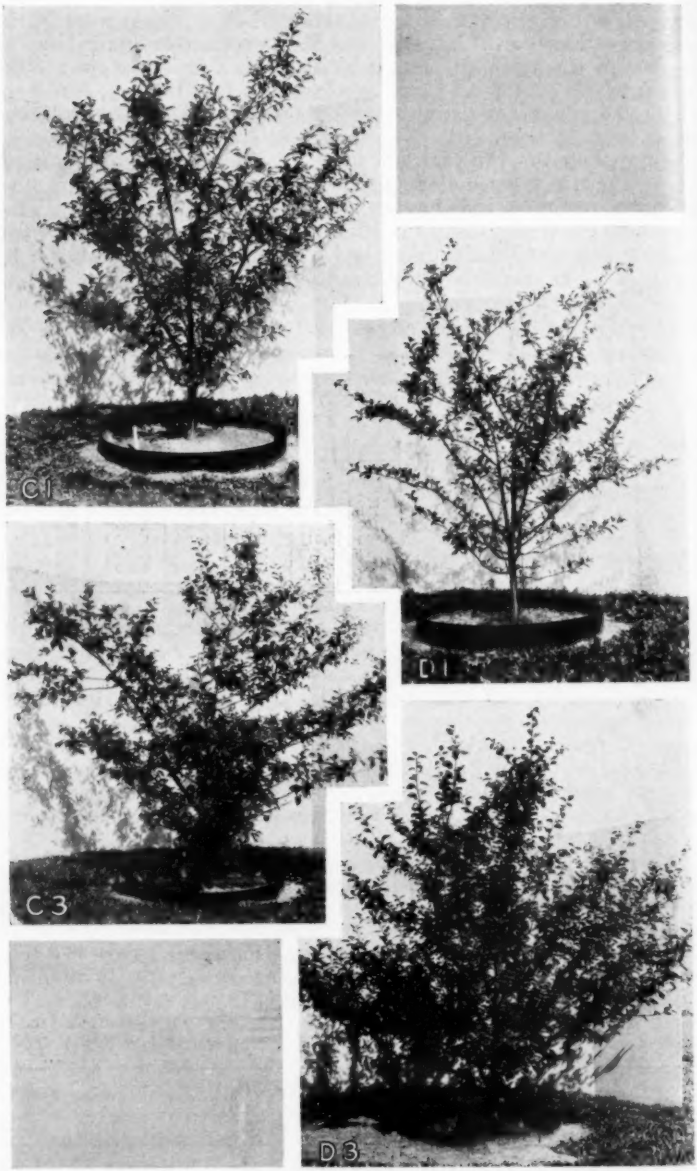

FIGURE 2.—Trees receiving phosphorus fertilizer only under cultivation, C1, and under sod, D1; also trees receiving a complete fertilizer under cultivation, C3, and under sod, D3. The roots of D3 escaped from the rim in 1926. Photographed September, 1927

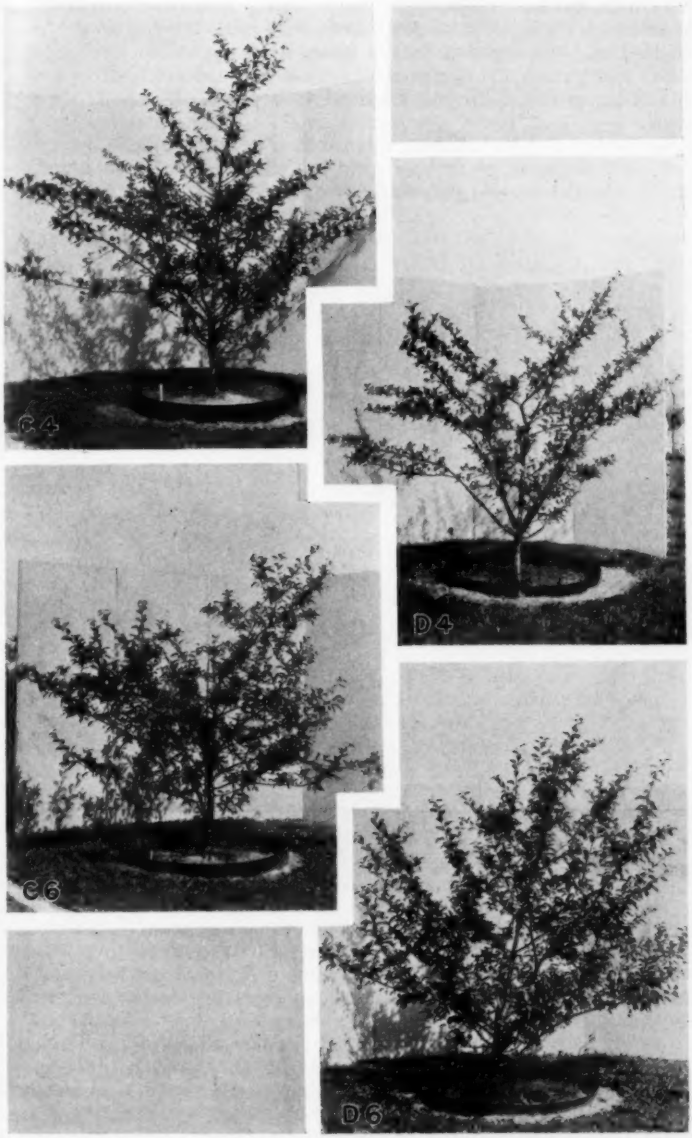

FIGURE 3.—Trees receiving no fertilizer under cultivation, C4, and under sod, D4; also trees receiving nitrogen and potassium only under cultivation, C6, and under sod, D6. Photographed September, 1927

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As soon as the leaves were harvested, the trees were cut off at a uniform height above the edge of the rims. The 1927 branch growth was measured and the total weight of each top secured. As the branch growth had been measured each year, it was possible to study total branch elongation for the years 1925, 1926, and 1927, during which the fertilizers could have influenced tree vigor. Table 7 gives this record.

Comparing sod and tillage, this table of branch elongation presents practically the same picture as table 6 in which leaf weights are shown, in sod without nitrogen growth decreased to a very pronounced degree;

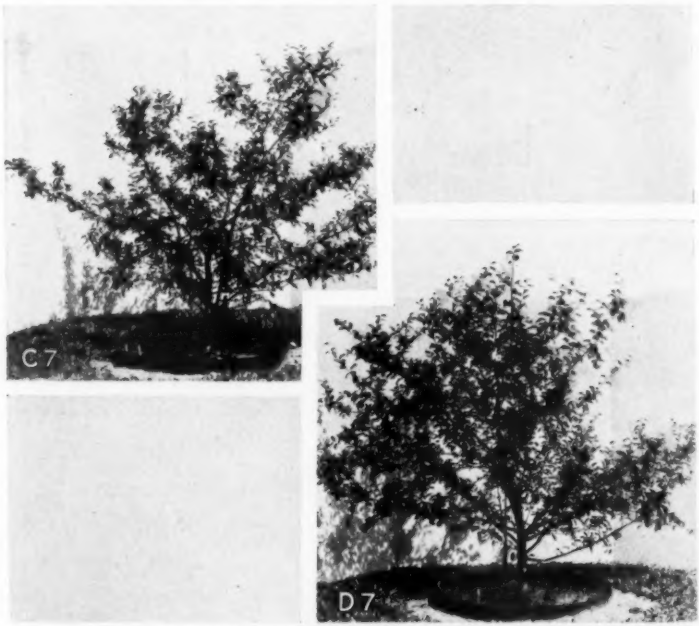

FIGURE 4.—Trees receiving nitrogen and phosphorus only under cultivation, C7, and under sod, D7. Photographed September, 1927

while the addition of nitrogen gave nearly equal growth under both sod and tillage. The one case among the trees receiving nitrogen that approached nearest to a significant difference between sod and tillage was in the nitrogen and phosphorus treatment, where the odds that sod was better than tillage were 20 to 1.

In order to make a statistical study of the effect of the fertilizers on total branch elongation, the records for each tree for each of the three years were used, thus giving nine measurements when either the three sod or the three tillage trees under one treatment were compared to those under a contrasted treatment. The presence or absence of nitrogen (N *v.* none; N, P, K *v.* P, K) results in such large differences, even with trees under cultivation, that statistical analysis is not necessary.

There is some evidence (Table 8) that the application of phosphorus stimulated tree growth. While the odds are not sufficiently large in all cases, the increase from 1925 to 1927 lends weight to this assumption. There is also some evidence that phosphorus gave a greater response under sod than under tillage.

Table 9 presents the results of treatments that differed only in the addition of potassium. There is no indication that this addition increased branch growth.

TABLE 8.—*Influence of phosphorus and potassium on tree growth expressed as odds that gain over contrasted treatment is significant*

Relative order of contrasted treatments	Odds for sod and tillage rims		Odds for total growth 1925-27	
	1925	1927	Sod	Tillage
PHOSPHORUS				
Row 1 (P) greater than row 4 (none)	* 4-1	47-1	12-1	12-1
Row 3 (N, P, K) greater than row 6 (N, K)	14-1	340-1	22-1	35-1
Row 5 (P, K) greater than row 4 (none)	1-1	36-1	41-1	19-1
Row 7 (N, P) greater than row 2 (N)	3-1	31-1	129-1	2-1
POTASSIUM				
Row 3 (N, P, K) greater than row 7 (N, P)	2-1	3-1	* 2-1	38-1
Row 5 (P, K) greater than row 1 (P)	2-1	* 5-1	* 4-1	* 2-1
Row 6 (N, K) greater than row 2 (N)	1-1	* 16-1	* 2-1	* 6-1

* Difference reversed.

TRUNK-DIAMETER INCREASE

The extent to which trunk-diameter measurements are used in interpreting orchard experiments makes it desirable to present these measurements for the rim trees. The coefficient of correlation between trunk-diameter increase during the period of fertilization and total branch elongation during the same period is 0.87 ± 0.026. The correlation between trunk diameter and weight of top is 0.88 ± 0.023.

A statistical study of percentage increase in trunk diameter from 1924 through 1927, the period of differential treatment, shows that this method of measuring trunk increase is not as satisfactory as the use of the actual gain or the final diameter. Some of the trees that were largest at planting and also at digging showed a smaller percentage increase with the same treatment than some that were much smaller both at planting and digging.

The use of Student's method to group trees of similar age or of the same initial vigor for comparison removes some of the difficulties caused by such variables as those encountered in the study of percentage trunk increase. The method of pairing adopted here grouped trees by age, and to a considerable extent by initial vigor, because of the method of planting (Table 1); but even this has not decreased the degree of variation sufficiently to make it desirable to use percentage increase.

Table 9 gives the trunk-diameter measurements taken when the trees were dug in 1927; Table 10 gives the actual diameter increase from July, 1924, to September, 1927. The very high correlation

(0.87 ± 0.026) between trunk measurements and branch elongation would lead one to expect the same conclusions from Tables 9 and 10 as from the tables showing branch growth, and such is the case. The results from the use of nitrogen are nearly the same with sod as with tillage, with the possible exception of the N, P treatment, where the trees growing in sod have the larger diameter. The odds for the significance of this difference in Table 9 are less than 4 to 1; and in Table 10, about 17 to 1; both too small to have much significance. Only one of the first 12 trees in Table 10 did not receive nitrogen; only one of the lowest 12 did receive nitrogen.

TABLE 9.—*Trunk diameter of apple trees, September, 1927*

Row	Treatment	Trunk diameter (millimeters) of trees in sod rim indicated				Trunk diameter (millimeters) of tree in tillage rim indicated			
		F	E	D	Average	C	B	A	Average
No. 1	P	57	58	58	57.7	69.	71	66	68.7
No. 2	N	68	65	71	68.0	66	88	75	76.3
No. 3	N, P, K	68	74	90	77.3	81	77	75	77.7
No. 4	None	41	61	61	54.3	65	68	63	65.3
No. 5	P, K	52	59	60	57.0	65	60	68	64.3
No. 6	N, K	71	69	73	71.0	74	73	70	72.3
No. 7	N, P	68	79	84	77.0	66	77	70	71.0

TABLE 10.—*Trunk-diameter increase of apple trees between July, 1924, and September, 1927*

Row	Treatment	Increase of diameter (millimeters) of trees in sod rim indicated				Increase in diameter (millimeters) of trees in tillage rim indicated			
		F	E	D	Average	C	B	A	Average
No. 1	P	[a] 28	20	20	23	32	[a] 28.5	36	32
No. 2	N	38	29	37	35	25	[a] 50	44.5	40
No. 3	N, P, K	38	36	[a] 50	41	44	39	46	43
No. 4	None	12	21	20	18	27	28	32.5	29
No. 5	P, K	23	20.5	20	21	32	20	42	31
No. 6	N, K	41	30	33	35	35	31	40	35
No. 7	N, P	42	44	45	44	29	30	40.5	33

[a] Roots escaped from rims 2 years before digging.

TOTAL WEIGHT OF TOPS AND ROOTS AT DIGGING

When the trees were dug, the trunk of each was cut off as close as possible to the place where the top had been grafted on the root. The soil was dug in three layers—0 to 7 inches, 7 to 21 inches, and 21 inches to the bottom. Each layer was spread on canvas and the roots separated from the soil and from the crown. (Table 11.) The trees that received no nitrogen had more roots by weight in the upper 7-inch layer. This layer was filled with very fine roots quite uniformly distributed. In rims receiving similar treatments the weight of the roots in the top layer was approximately the same under sod and under tillage. In the second layer (7 to 21 inches) there were more of the larger roots, and a larger proportion nearer the rims. In this layer the trees receiving nitrogen had many more

roots than those not receiving it; among the latter the tilled trees had a larger quantity of roots than the untilled.

The roots from 21 inches down to within 2 or 3 inches of the bottom were mainly of finger size. Many of these were against the rim. Near the crushed limestone the fine feeding roots again became prominent.

Table 12 gives the total weight of the roots for each tree. When the tops of the trees were cut off, each main branch was removed separately and its diameter and weight recorded. Table 13 gives the total of these weights for each tree. Comparing these for root weight and top weight, we find a rather close agreement. All but 2 of the 12 trees highest in root weight are also among the 12 highest in top weight, and all but 2 of the 12 lowest in root weight are among the 12 lowest in top weight. Two trees not receiving nitrogen are among the highest 12 in root weight, and 1 receiving nitrogen is among the lowest 12. One tree of the highest 12 in top weight received no nitrogen; none of the lowest 12 received any nitrogen.

TABLE 11.—*Distribution of apple-tree roots as measured by average weight per tree at different ground levels*

Depth from surface (inches)	Weight (grams) of roots from trees that received—			
	No nitrogen		Nitrogen	
	Sod	Tillage	Sod	Tillage
Crown	1,576	2,559	2,741	3,000
0 to 7	1,828	2,161	1,067	1,018
7 to 21	1,723	4,056	5,390	5,531
Below 21	1,569	1,819	2,295	2,672

TABLE 12.—*Total weight of apple-tree roots at digging time*

Row	Treatment	Weight (grams) of roots of trees in sod rim indicated				Weight (grams) of roots of trees in tillage rim indicated			
		F	E	D	Average	C	B	A	Average
No. 1	P	6,725	7,895	7,450	7,357	12,275	13,175	11,135	12,195
No. 2	N	11,845	10,366	10,110	10,774	10,300	14,805	13,042	12,716
No. 3	N, P, K	9,420	13,040	16,915	13,125	12,395	13,675	13,315	13,128
No. 4	Check	5,215	9,950	7,350	7,505	10,870	11,790	9,140	10,600
No. 5	P, K	8,195	8,325	6,815	7,778	10,695	7,930	8,615	9,080
No. 6	N, K	9,935	11,090	10,610	10,545	11,700	10,705	10,110	10,838
No. 7	N, P	9,905	11,950	12,730	11,528	13,075	12,260	11,275	12,203

TABLE 13.—*Weight of apple-tree tops at digging time*

Row	Treatment	Weight (grams) of tops of trees in sod rim indicated				Weight (grams) of tops of trees from tillage rim indicated			
		F	E	D	Average	C	B	A	Average
No. 1	P	7,745	6,710	9,495	7,983	10,270	16,300	10,410	12,327
No. 2	N	12,960	9,685	12,410	11,685	10,490	21,205	16,875	16,190
No. 3	N, P, K	9,710	13,960	26,910	16,860	13,820	17,215	17,655	16,230
No. 4	Check	4,195	7,510	7,625	6,443	9,745	9,660	9,210	9,538
No. 5	P, K	6,785	7,550	6,790	7,042	10,950	7,230	10,960	9,713
No. 6	N, K	11,330	11,670	14,410	12,470	11,765	11,680	10,570	11,338
No. 7	N, P	13,685	13,980	17,265	14,977	13,455	16,550	13,360	14,455

The significance of gains or losses is more apparent from an inspection of Table 14, which gives the combined top and root weight for each tree. A study of the distribution of the heaviest trees shows that

all but 1 of the first 12 received nitrogen. The roots of this tree, B–1, probably escaped from the rim in 1926, which may account for the greater growth of this tree. Eight of the highest were in the cultivated half of the area. The twelfth lowest tree received a complete fertilizer; the other 11 lowest trees received no nitrogen. Ten of the lowest 12 were in sod.

The criticism may be made, with justice, that in combining trees of two ages in the same ranking the younger trees may be handicapped. The younger trees planted in 1923 were nearly twice as heavy at planting as those planted in 1922. Their growth was very satisfactory during the preliminary period. There are some indications that the rims checked the growth of the older trees during 1927 to an extent sufficient to permit the younger trees to gain on the older ones, but not to catch up with them, especially in the sod block. None of the sod trees ranking highest in Tables 12 and 13 was in the younger group.

The fact that there are trees of two ages in each treatment does not affect the results when Student's method is used in determining the significance of gains or losses; by this method, trees of the same age standing side by side are compared. Table 15 shows the odds of the significance of the gain of one treatment over another both for the whole row and for those parts in sod and in tillage, based on the total weight of tops and roots.

TABLE 14.—*Total weights of apple-tree tops and roots at digging time*

Rows	Treatment	Weight (grams) of tree tops and roots in sod rims as indicated				Weight (grams) of tree tops and roots in tillage rims as indicated			
		F	E	D	Average	C	B	A	Average
No. 1	P	14,470	14,605	16,945	15,340	22,545	29,475	21,545	24,522
No. 2	N	24,805	20,051	22,520	22,459	20,790	36,010	29,917	28,906
No. 3	N, P, K	19,130	27,000	43,825	29,985	26,215	30,890	30,970	29,358
No. 4	None	9,410	17,460	14,975	13,948	20,615	21,450	18,350	20,138
No. 5	P, K	14,980	15,875	13,605	14,820	21,645	15,160	19,575	18,793
No. 6	N, K	21,265	22,760	25,020	23,015	23,465	22,385	20,680	22,177
No. 7	N, P	23,590	25,930	29,995	26,505	26,530	28,810	24,635	26,658

TABLE 15.—*Influence of treatment on total weight of apple trees expressed as odds that one treatment is better than another*

Relative order of contrasted treatments	Odds for—		
	Tillage rims	Sod rims	Both combined
Row 1 (P) better than row 4 (none)	13–1	2–1	8–1
Row 2 (N) better than row 4 (none)	10–1	12–1	98–1
Row 3 (N, P, K) better than row 4 (none)	43–1	14–1	150–1
Row 4 (None) better than row 5 (P, K)	2–1	[a] 1.6–1	1.2–1
Row 6 (N, K) better than row 4 (none)	28–1	45–1	69–1
Row 7 (N, P) better than row 4 (none)	11–1	77–1	893–1
Row 1 (P) better than row 5 (P, K)	5–1	1.6–1	7–1
Row 2 (N) better than row 6 (N, K)	6–1	[a] 1.5–1	5–1
Row 3 (N, P, K) better than row 7 (N, P)	6–1	2–1	6–1
Row 7 (N, P) better than row 2 (N)	[a] 4–1	6–1	1.7–1
Row 3 (N, P, K) better than row 6 (N, K)	22–1	4–1	31–1
Row 7 (N, P) better than row 6 (N, K)	42–1	42–1	1,428–1
Row 3 (N, P, K) better than row 2 (N)	1.2–1	3–1	4–1
Row 2 (N) better than row 1 (P)	4–1	41–1	105–1
Row 2 (N) better than row 5 (P, K)	7–1	40–1	74–1
Row 7 (N, P) better than row 1 (P)	6–1	168–1	199–1
Row 6 (N, K) better than row 5 (P, K)	8–1	52–1	142–1
Row 3 (N, P, K) better than row 5 (P, K)	23–1	9–1	90–1
Row 7 (N, P) better than row 5 (P, K)	16–1	50–1	555–1

[a] Comparison is reversed.

By including the six trees receiving common treatment under both sod and tillage in a single plot, as shown in the last column of Table 15, all plots receiving nitrogen alone or in combination made significant gains in total weight of tops and roots over the untreated plot; while phosphorus alone or in combination with potassium failed to give significant increases over the untreated plot.

RELATIVE VALUE OF PLANT FOODS

There are six cases in Table 16 in which nitrogen, either alone or in combination with other elements, may be compared with nonnitrogenous fertilizers (N *v.* P; N *v.* P, K; N, P *v.* P; N, K *v.* P, K; N, P, K *v.* P, K; N, P *v.* P, K). In every case the gain from the use of nitrogen is significant when the results with sod and tillage are combined.

In the three comparisons which differed only by the presence or absence of potassium (P *v.* P, K; N *v.* N, K; N, P *v.* N, P, K), the use of potassium resulted in no significant increase in total weight. When these three treatments were combined, making 18 pairs that differ only in the presence or absence of potash, there was still no significant difference in weight.

In the three comparisons in which phosphorus was the variable (check *v.* P; N *v.* N, P; N, K *v.* N, P, K) it was only when the phosphorus was added to the other two elements that a significant gain was secured. When nitrogen alone was added to each element phosphorus caused a much greater gain than the potassium (N, P *v.* N, K). When the three phosphorous treatments were combined in a single comparison, the 18 pairs in sod and tillage together gave odds of 81 to 1 that there was a significant increase in the case of the trees receiving phosphorus.

Past experience has emphasized the importance of studying any fertilizer combination under both sod and tillage separately. When this comparison was made with the trees in the rims, the number of pairs available was reduced from six to three. In general, the use of smaller numbers makes it necessary to obtain larger differences in order to secure the same significance for gains or losses.

When only the three trees were compared, the N, K treatment showed practically significant gains as contrasted with the untreated trees under both sod and tillage; the N, P treatment gave a significant gain over the untreated trees under sod only; and the N, P, K treatment compared to no treatment was significantly different only under tillage. The use of nitrogen alone failed to produce significant increases in either sod or tillage. The high degree of variability and the small number of pairs available caused these decreased odds. This was particularly true in comparisons involving tree D–3, the large weight of which led to such a high standard deviation in this block that the odds were seriously decreased. On the other hand, the trees in the row receiving the N, K treatments were very uniform, and comparisons with this row often gave high odds.

The six cases in which nitrogen was compared to nonnitrogenous fertilizers gave significant increases when the results from sod and tillage were combined; but when these were separated, the only significant increases observed were where nitrogen was used in sod. Even here the use of a complete fertilizer failed to show a significant

increase over the combination of phosphorus and potassium. This failure, again, was probably due to the abnormal performance of tree D–3.

These results are very similar to those secured under field conditions with this same soil (*1*, *2*). In a study of contrasting fertilizer treatments carried on for 21 years in a cultivated orchard it was found that the results from plots treated with phosphorus and potassium were no better than those from untreated plots; but when nitrogen was added to phosphorus alone or to both phosphorus and potassium there was an increase in cover-crop growth, tree growth, and yield. It is probable that the better returns from phosphorus alone in the rims as compared to the P, K treatment in the field are due to the higher organic content in the rim soil where either a sod was grown or a considerable quantity of chopped rye added.

WEIGHT OF FRUIT

As soon as the buds began to unfold in 1926, it was apparent that some of the older trees were carrying a considerable number of blossom clusters. These were more numerous on the most vigorous trees. Since permitting trees of this age to mature a crop might check their growth to such an extent as to influence the final results, all blossom clusters were removed in the early pink stage. Table 16 shows the number removed from each tree.

When the buds began to open in 1927, many of the trees were overloaded with bloom, and the blossom clusters were accordingly thinned to a stand approximately 6 inches apart, by pinching out the excess blossom clusters as soon as they were sufficiently expanded to be reached easily. Table 16 shows the number of clusters removed. It should be remembered that the outer rows of trees were a year younger than the inner four.

TABLE 16.—*Blossom clusters removed from apple trees in 1926 and 1927*

BLOSSOM CLUSTERS REMOVED, 1926

Row	Treatment	Number of blossoms removed per tree in sod rims indicated			Number of blossoms removed per tree in tillage rims indicated		
		F	E	D	C	B	A
No. 1	P	0	0	0	2	0	11
No. 2	N	0	0	21	17	50	4
No. 3	N, P, K	3	227	98	114	175	45
No. 4	None	0	0	0	0	5	1
No. 5	P, K	0	0	0	10	9	9
No. 6	N, K	0	70	32	7	9	1
No. 7	N, P	4	42	99	87	264	2

BLOSSOM CLUSTERS REMOVED TO THIN TO 6 INCHES, 1927

No. 1	P	0	0	0	118	50	132
No. 2	N	0	123	340	113	242	118
No. 3	N, P, K	165	500	577	500	542	395
No. 4	None	0	0	0	0	0	32
No. 5	P, K	0	0	0	200	75	84
No. 6	N, K	0	309	270	335	275	0
No. 7	N, P	173	352	600	722	830	237

Table 16 shows that trees under tillage blossomed somewhat earlier and considerably more heavily than trees in sod receiving the same fertilizer treatment. The growth records also show how closely in this instance blossoming was related to tree vigor. It is probable that this growth relation is the cause of the decreased blooming of trees in the sod, and also of the greater bloom on trees receiving nitrogen and phosphorus.

Table 17 gives the total weight of fruit on the trees in 1927. Because of the irregular water supply, most of the fruit dropped to the ground just as it was maturing. The weights given include the drops. The rank in fruit yield is not quite the same as in the number of clusters removed in the spring. Part of the irregularity is due to some rather severe spray burning that injured the clusters which were left. For this reason, and because the record is for a single year only, not much importance should be attached to yields in interpreting the results of this experiment. In general, it may be said that the trees receiving nitrogen and phosphorus and those receiving nitrogen, phosphorus, and potassium bloomed most heavily; but that the trees receiving only nitrogen or nitrogen with potassium made a relatively heavier set of fruit.

TABLE 17.—*Total weight of apples picked and dropped from trees, 1927*

Row No.	Treatment	Weight of fruit (grams) from trees in sod rims indicated				Weight of fruit (grams) from trees in tillage rims indicated			
		F	E	D	Average	C	B	A	Average
1	P	230	0	0	77± 42	590	800	7,240	2,877±1,203
2	N	1,260	5,590	5,305	4,052± 771	1,880	9,060	4,865	5,268±1,148
3	N, P, K	1,230	2,280	7,415	3,642±1,053	910	6,375	5,750	4,345± 952
4	None	0	0	0	0	0	180	320	167± 12
5	P, K	0	0	0	0	3,880	1,710	1,530	2,373± 416
6	N, K	440	9,930	9,650	6,673±1,661	5,220	5,185	2,660	4,355± 467
7	N, P	980	225	4,730	1,978± 768	7,130	2,230	7,883	5,748± 977
					2,346± 605				3,590± 455

CORRELATIONS AMONG GROWTH AND YIELD RECORDS

There are close correlations among the growth measurements. The treatments which gave the most branch growth also gave the largest trunk diameters and the greatest weights of the trees. Likewise, the individual trees showing the most branch growth also showed the largest trunk diameters and total weights.

Table 18 gives the correlations among the growth measurements studied. In each case the correlation is very high, over 0.8. The high correlation of the various growth measurements with trunk-diameter measurement is especially important because of the frequency with which this measurement is used as an index of growth in orchard experiments. Furthermore, the increase in trunk diameter during the period of fertilization is closely correlated with branch elongation during the same period. A study of the correlations with weight, which must be taken as the absolute measurement of growth, shows that both top weight and total weight are closely correlated with trunk diameter, and top weight is just as closely correlated with branch elongation.

TABLE 18.—*Correlations of apple-tree growth records*

Measurements correlated	Correlation*
Trunk diameter with total weight	0.87±0.025
Trunk diameter with weight of tops	.88± .023
Trunk diameter with total branch elongation	.83± .032
Trunk diameter increase, 1924 to 1927, with branch elongation, 1925 to 1927	.87± .026
Branch elongation with weight of tops	.88± .023

* In biometrical studies a correlation factor of over 0.5 is considered certain correlation, provided that the correlation factor is over six times the probable error. If the correlation factor is less than 0.3 or is less than six times the probable error, there is no evident correlation.

Table 19 gives the correlations of yield and blossom records with the growth measurements taken. It is not safe to generalize on the basis of the yields of one year. As the records stand, the correlations of yield with growth measurements are not nearly so high as those of the growth measurements among themselves. Yield is most closely correlated with total weight; even here the correlation is only 0.55, which figure compares unfavorably with correlations of more than 0.8 among the growth records.

TABLE 19.—*Correlations of apple-tree yield and blossom records with growth records*

Items correlated	Correlation
Yield with trunk diameter	0.48 ±0.080
Yield with total weight	.55 ± .072
Yield with weight of tops	.53 ± .075
Yield with total branch elongation	.45 ± .083
Yield with branch elongation, 1925 to 1927	.38 ± .089
Yield with branch elongation of 1926	.28 ± .096
Yield of nitrated trees with 1926 branch elongation of nitrated trees	.16 ± .130
Yield with 1927 branch elongation	.24 ± .098
Yield of nitrated trees with 1927 branch elongation of nitrated trees	−.047± .137
Yield with blossom clusters removed, 1927	.42 ± .086
Blossom clusters removed, 1927, with total weight	.57 ± .070
Blossom clusters removed, 1927, with weight of tops	.56 ± .071
Blossom clusters removed, 1927, with trunk diameter	.58 ± .069
Blossom clusters removed, 1927, with total branch elongation	.62 ± .064
Blossom clusters removed, 1927, with branch elongation, 1925 to 1927	.36 ± .091

There is little or no evidence of correlation between yield and branch growth of the previous year or of the same year. Since the 1927 yield shows a fair correlation with total branch growth, but not with the growth of 1926 and 1927, the correlation must be with the earlier growth. The roots of those trees that made a good growth during the early years probably had so completely occupied the soil of the rims that by 1926 root crowding had begun to check their growth. The checking of growth of these vigorous trees created the right conditions for fruit production.

There is a rather low correlation between the yield of 1927 and the number of blossom clusters removed in order to thin them to 6 inches. Spray injury probably accounts for some of this low correlation. The thinned clusters were proportional to the actual number of clusters present on the tree. The number of clusters removed gives a fair correlation with total tree weight, which is also the measurement showing the highest correlation with yield. The number of clusters removed shows an even greater correlation with total branch elongation, though not with branch growth during the period of fertilization.

Thus it seems that the amount of bearing surface, as indicated by total branch growth, is an important factor in blossom production.

SUMMARY AND CONCLUSIONS

The use of metal cylinders or rims as a means of studying the effect of different fertilizer treatments on apple trees proved valuable in hastening the fertilizer responses.

The escape of roots from certain rims increased the variability in the growth of the trees, and consequently decreased the accuracy with which the results could be interpreted. However, this decreased accuracy did not destroy the value of the results.

There is a very high correlation—over 0.8—among the growth factors studied, namely, weight of tops, weight of roots, trunk diameter, and branch elongation. The studies of blossom production, yield, and tree growth indicate that blossom production is most highly correlated with total branch elongation and bearing surface, but that, in these trees, yield has a somewhat lower correlation with tree growth. The quantity of fruit produced is only partly dependent on the number of blossoms.

In general, the results from the use of fertilizers confirm the results of previous research in the orchard. Sod, without the addition of nitrogen, checked the growth of the trees very seriously, even when potassium and phosphorus were applied. However, when nitrogen (expecially nitrogen in combination with phosphorus) was applied to trees growing in sod, the growth was nearly as good as that of cultivated trees receiving the same fertilizer.

Trees that were cultivated but received no nitrogenous fertilizer were not as vigorous as those that were cultivated and also received nitrogen.

When phosphorus alone was applied to trees growing in sod, the growth was noticeably greater than that of the untreated check. The addition of phosphorus to nitrogen did not produce a definitely better growth than nitrogen alone; but when phosphorus was added to nitrogen and potash, in sod, growth increased.

The addition of potassium to nitrogen or phosphorus or to both did not modify the growth of the trees but may have increased the setting of the fruit.

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THE EFFECT OF YEAST AND CASEIN SUPPLEMENTS TO CORN AND SOYBEAN RATIONS WHEN FED TO RATS AND SWINE[1]

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INTRODUCTION

One of the problems of the corn-producing area has been the inadequate supply of efficient protein concentrates for livestock. Because of an increasing acreage in soybeans (*Soja max*), in the Middle West, this seed has received attention as a possible protein supplement for corn in animal rations. A large number of feeding experiments with farm animals in which soybeans or soybean products were used have demonstrated the feeding value of this concentrate. In general the impression is created that soybeans, at least in the raw condition, have a limited supplementing value in rations for certain classes of livestock.

The analyses of Street and Bailey (*7*)[2] show the soybean to be high in protein and fat and low in ash and carbohydrates. The protein values found in these analyses ranged from 36.8 to 45.5 per cent.

The high protein content of soybeans led Osborne and Mendel (*4*) to investigate their biological character. They fed corn gluten and soybean meal to rats and found that when the meal furnished 4.5 per cent protein in combination with 11.4 per cent protein from corn gluten satisfactory growth resulted. They attributed the supplementing action of soybean protein to its content of lysine and tryptophane, which are lacking in corn gluten.

Osborn and Mendel (*5*) also investigated the soybean as the sole source of protein for the rat and reported that when the diet was complete in other respects it was adequate for normal growth. They found that soybeans furnished a fair supply of "fat soluble vitamin" and an adequate quantity of "water soluble vitamin." However, their data indicated that soybeans were deficient in mineral matter, especially calcium and chlorine. They also noted that soybeans were not readily consumed by rats, presumably because of the disagreeable taste they possessed.

The analyses of Jones and Waterman (*3*) and Daniels and Nichols (*1*) show soybean protein to contain a variety of amino acids, some of which are essential for growth. Jones and Waterman reported a tryptophane content of the soybean protein glycinin as 1.37 per cent and of lysine as 9.06 per cent.

It appears, then, from chemical and biological analyses that soybeans should be a good supplement for protein-deficient grains in livestock rations.

[1] Received for publication July 22, 1931, issued April, 1932.
[2] Reference is made by number (italic) to Literature Cited p. 274.

EXPERIMENTAL PROCEDURE

Rats and swine were used as experimental animals in this study. The rats were selected from the stock colony at 24 to 30 days of age, at which time they generally weighed between 35 and 45 g. They were quartered in a steam-heated animal house in individual false-bottom wire cages. A modified McCollum feed cup was used, and the food consumed was determined weekly. Two male and two female animals constituted a test group. Each ration was tested on at least three groups. All the rats used in the experiments received daily irradiations from a quartz mercury vapor lamp for 14 minutes.

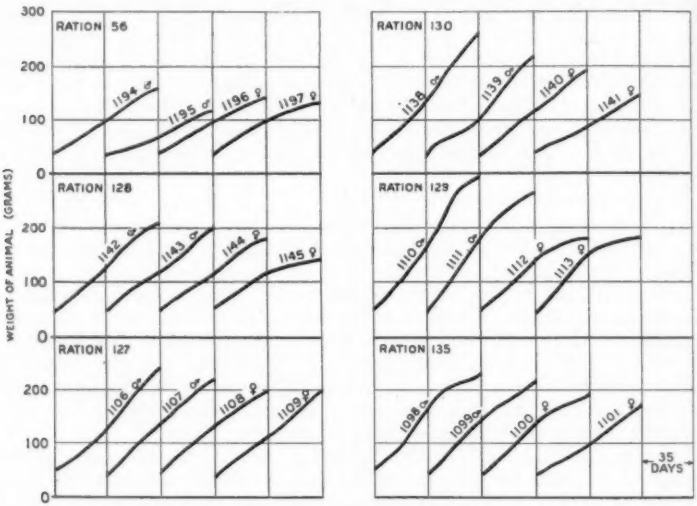

FIGURE 1.—Growth of rats on various experimental rations. Slow growth was obtained on the corn-soybean-mineral ration No. 56. Increased growth was apparent on rations 127 and 128, in which 5 and 2.25 per cent casein protein had been added to the basal ration. When the soybeans in rations 127 and 128 were replaced by corn, as in Nos. 129 and 130, growth improved still further. Growth was also observed when the soybeans of the basal ration were cooked, as in No. 135. See Table 1 for composition of the rations

Distilled water was kept before them at all times and was fresh daily. The length of the experimental period was 10 weeks.

The pigs were quartered in dry lots at the experimental swine farm. Each test lot contained 10 pigs of comparable age and weight. The pigs were all either high-grade or purebred Duroc Jersey stock raised on the swine farm. These animals were weighed at 10-day intervals. Food consumption records were obtained from each lot. The experimental period was 70 days.

The basal ration (ration 56) had the following composition: Yellow corn, 84 per cent; soybeans,[3] 14 per cent; salt mixture,[4] 2 per cent.

The experimental rations differ from No. 56 by the substitution of protein concentrates and vitamin B carriers for a part of the corn-soybean mixture. In some trials the soybeans were cooked and in others they were left entirely out of the mixture.

[3] Manchu variety.
[4] Composed of limestone 100 parts, special steam bone meal 100 parts, salt 10 parts.

EXPERIMENTAL RESULTS WITH RATS

GROWTH ON A PROTEIN-DEFICIENT DIET

Inspection of Figure 1 shows that growth on the basal ration (No. 56) was slow. This subnormal rate of gain was obtained in 13 trials on this ration. In no instance did any group of animals in this series approach satisfactory growth. The average total gain per animal was 63.7 gm. for the slowest growing lot and 121.5 gm. for the fastest.

Marked variation in individual growth rates occurred, which is an indication of an inadequate diet. A few individuals grew at a fairly rapid rate, although the majority gained slowly. These results show beyond question that the basal ration is inadequate for satisfactory growth of rats. In attempting to determine the cause of this inadequacy the protein moiety of the mixture was studied. Since the introduction of a larger proportion of soybeans in the ration would render it less desirable for swine, because of the tendency of soybeans to form soft pork, this constituent was not increased in these trials. However, in earlier work a quantity of soybeans furnishing an additional 5 per cent of crude protein was added to the basal mixture without favorable results.

Previous work with rats indicated that the ration might be deficient in total protein. It is generally believed that rations carrying less than 14 to 15 per cent of protein are deficient; the experience of the writers, however, does not entirely confirm this view. By feeding corn and casein diets with a protein level of only 13.6 per cent excellent growth was obtained. (Fig. 1, ration 129.) Moreover, a large number of swine rations containing less than 15 per cent protein produced satisfactory results. Nevertheless, it seemed that the basal ration could be improved by the addition of protein. Then, too, there might be some qualitative deficiency in the mixture that could be corrected. Studies were instituted with this in mind.

GROWTH ON A DIET WITH PROTEIN ADDITIONS

In the earlier work, tankage, meat and bone scrap, and casein were used as supplements. These were found to have a fairly comparable nutritive value, but since casein gave slightly better results it was selected for the work reported here. The casein was incorporated in the corn-soybean-mineral ration in amounts furnishing 2.25 and 5 per cent of crude protein. Table 1 gives the protein content of the rations.

TABLE 1.—*Composition of experimental rations*

Ration No.	Yellow corn	Soybeans	Cooked soybeans[a]	Casein	Yeast	Mineral No. 6 [b]	Total protein
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
56	84.0	14.0	----	----	----	2.0	13.4
127	78.3	13.0	----	6.7	----	2.0	17.5
128	81.4	13.6	----	3.0	----	2.0	15.2
129	91.3	----	----	6.7	----	2.0	13.6
130	95.0	----	----	3.0	----	2.0	11.3
131	81.4	13.6	----	----	3.0	2.0	14.6
132	75.6	12.7	----	6.7	3.0	2.0	18.7
133	78.8	13.2	----	3.0	3.0	2.0	16.5
135	84.0	----	14.0	----	----	2.0	13.4

[a] Cooked in steam until soft, and dried.
[b] Mineral mixture No. 6: Limestone, 100 parts; special steam bone meal, 100 parts; salt, 10 parts.

Reference to Figure 1 (rations 127 and 128) shows a marked increase in rate of gain when either the high or the low level of casein was employed. Since more favorable growth resulted from the use of the higher level of casein it would appear that, in part, the quantity of protein was an important factor.

The question arose as to whether the increased growth exhibited by the animals on the lower level of casein was due to a true supplementing action of casein for soybean protein, or to a building up of the total protein content of the ration, or to other factors. In order to determine this point animals were placed on a ration of corn and mineral, with casein furnishing 2.25 per cent protein. (Ration 130.) Here the soybeans of ration 128 were replaced by an equal amount of corn. If the favorable response that was obtained by the use of ration 128 (composed of corn, soybeans, casein, and mineral) was due to a supplementing action of casein for soybeans, then growth on the modified ration (No. 130), in which soybeans were replaced by corn, should be at a slower rate. The results of this trial, presented in Figure 1 (rations 128 and 130), show that the growth of the animals was as good when soybeans were omitted as when they were present, and in some instances even better. The same results were obtained when soybeans were replaced by an equal amount of corn in the ration containing the higher level of casein protein. (Ration 129.)

There is no evidence from these experiments that soybean protein was supplemented by the addition of casein. It appears that the protein of soybeans is not utilized above a certain point, owing possibly to factors contained in the soybean, the nature of which is not known. That is, when casein was added to the basal corn-soybean mixture better growth resulted probably not because the ration was qualitatively or quantitatively different from the original ration but rather because the nutrients of casein were utilized readily to supply the deficiencies of corn, whereas those of soybeans were not utilized.

Although casein supplements corn alone, it is indicated from these experiments that in so far as improving the utilization of soybeans in combination with corn is concerned, the addition of casein protein, as a supplement, is of questionable value.

GROWTH ON DIETS CONTAINING COOKED OR UNCOOKED SOYBEANS

Since the work did not reveal a qualitative inadequacy of soybeans but rather an inability of the animal organism to utilize the potential nutritive value in them, it was decided to process the beans in several ways before feeding. The work described is concerned with heating, especially cooking.

Osborne and Mendel (*5*) in 1917 showed that cooking improves the feeding value of soybeans for rats. Later, Robison (*6*) fed cooked undried soybeans to hogs as a supplement to corn with very satisfactory results. It seemed desirable to study the effect of cooking in more detail.

Soybeans were cooked by passing steam through them until they became soft. Then they were rapidly dried to approximately their original moisture content.[5] Soybeans prepared in this manner were

[5] Cooking and drying were done by the agricultural engineering department.

incorporated in the feed in the same ratio as the unprocessed beans. The results are presented in Figure 1. (Ration 135.)

Ration 135, containing cooked soybeans, has a crude protein content of 13.4 per cent. For comparative purposes reference may be made to ration 129, containing approximately the same protein content derived from corn and casein.

Growth on the cooked soybean ration was definitely superior to that obtained with raw soybeans. Moreover, it compared favorably with that obtained by feeding a ration of corn and casein of about the same protein level, although the latter combination was somewhat superior. These results point to the conclusion that soybean protein is not deficient from a qualitative standpoint.

Investigations are being made to determine the cause of the improvement in the nutritive value of soybeans brought about by heating. It is unlikely that any of the major food constituents are changed in a qualitative manner. In an earlier paper [6] it was shown that the digestibility of the ration is not changed by cooking. Although the explanation of the greater nutritive value of cooked soybeans is not clear at this time, there is some reason for believing that certain materials of a toxic nature are removed or destroyed during the heating process.

GROWTH ON A DIET WITH YEAST ADDITIONS

In earlier trials the writers tried to improve growth by the addition of dried brewers' yeast to the ration on the assumption that it was deficient in the vitamin B complex, especially in the growth-promoting factor. The results of this study were so varied as to preclude the drawing of any definite conclusions. Osborne and Mendel (*5*) found soybeans to be an adequate source of "the water soluble vitamin." So far as the writers are aware, no experiments have been reported that demonstrate the relative amounts of vitamin B (B_1) and vitamin G (B_2) in soybeans. The writers have found [7] that a purified ration, complete except for vitamin B, must contain at least 12 per cent of soybeans in order to produce small weekly gains in the weight of rats. Since corn is rich in the antineuritic vitamin B (B_1) and poor in the growth-promoting factor (B_2), (*2*) there is no reason to believe that the ration used by the writers is deficient in the antineuritic vitamin, although it might be deficient in the growth-promoting factor. The work herein deals with the addition of 3 per cent yeast to the basal mixture, and the same supplemented with 2.25 and 5 per cent protein from casein. The results are presented in Figure 2.

The addition of yeast to the basal mixture consistently caused a slight but definite improvement in growth. (Ration 131.) However, the improvement was not great enough to be considered satisfactory. From the standpoint of improving corn-soybean rations it was concluded that yeast is of little, if any, value.

When yeast was added to rations containing casein the results were again irregular, confirming results obtained by the writers in earlier experiments.

[6] SHREWSBURY, C. L. THE NUTRITIVE VALUE OF SOYBEANS. THE EFFECT OF HEAT. Paper read before the Amer. Chem. Soc. Annual Meeting, Indianapolis, 1931.

[7] Unpublished data.

EXPERIMENTAL RESULTS WITH SWINE

All of the rations described above were fed to swine. Table 2 gives the data obtained.

The pigs weighed about 32 pounds at the beginning of the experiment. In earlier experiments heavier pigs were employed, but it was felt that more satisfactory information could be obtained in this study by the use of smaller animals. One of the writers [8] (*8*) has demonstrated that 100 to 110 pound pigs thrive on corn-soybean rations in dry lot. Lighter pigs will grow well on corn-soybean rations if allowed to forage legume pasture, but these lighter animals do not make satisfactory gains if fed in dry lot.

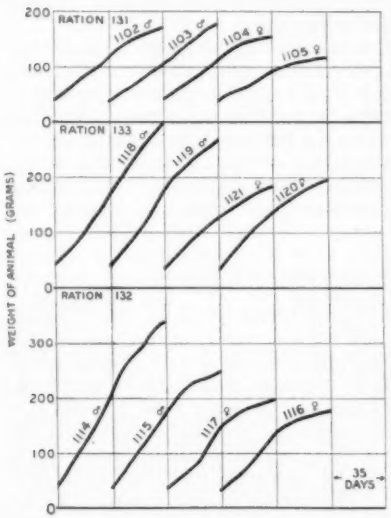

FIGURE 2.—Growth of rats on experimental rations to which yeast was added. Growth on ration 131 shows the effect of adding 3 per cent dried brewers' yeast to the basal diet; results should be compared with those for ration 56, Figure 1. Growth on rations 132 and 133 shows the effect of supplementing the basal diet with 3 per cent dried brewers' yeast and with 5 per cent casein protein in the case of 132, and 2.25 per cent in the case of 133; results should be compared with those for rations 127 and 128, Figure 1

Table 2 shows that the results obtained with swine were substantially the same as those described above for rats. The most striking feature of this experiment was the behavior of the lots on the basal corn-soybean-mineral ration (lot 1) and that on the same ration prepared with cooked soybeans (lot 10).

The pigs fed the basal ration grew very slowly (0.31 pound per day). These animals were all in good condition at the beginning of the experiment, but in a few weeks they became very unthrifty. The variability among individuals was pronounced. Some gained fairly well but the majority remained almost stationary, and a few lost weight. The hair coat in this lot was poor and shaggy, and the animals were humped and thin. However, when cooked soybeans replaced uncooked soybeans the situation was reversed. The pigs made an average daily gain of 1 pound and all were fat and had good coats of hair. Those that were smallest at the start grew rapidly and remained thrifty throughout the feeding period, while the smaller pigs receiving the raw soybean ration remained small and their condition became poorer as the experiment proceeded.

While too much emphasis should not be placed on feed consumption in the case of these pigs, because of the tendency of all the groups to waste feed at the self-feeders, it is interesting to note the greater utilization of feed by the pigs on the ration containing cooked soybeans. Although they made an average daily gain of more than three

[8] VESTAL, C. M., SOYBEANS FOR HOGS, Ind. Agr. Expt. Sta. Hog Summary Leaflet H-8. 1924. [Mimeographed.]

times that of pigs fed uncooked soybeans, they required only about half as much of feed to produce a unit of gain.

TABLE 2.—*Data obtained when the experimental rations were fed to swine*

[Ten pigs in each lot]

Lot No.	Ration No. [a]	Average daily feed consumption	Feed required to make 100 pounds gain	Average daily gain	Average gain
		Pounds	*Pounds*	*Pounds*	*Pounds*
1	56	2.12	686.3	0.31	27.8
2	127	2.77	402.4	.69	62.0
3	128	2.61	414.6	.63	56.6
4	129	4.16	344.8	1.21	108.5
5	130	3.01	476.4	.63	56.9
6	131	2.35	550.0	.43	38.4
7	132	3.46	385.2	1.03	93.0
8	133	2.73	373.2	.73	65.8
10	135	3.45	345.3	1.00	89.9

[a] See Table 1 for composition of rations.

When casein protein was added (lots 2 and 3) the gains were greater than on the unsupplemented ration. When corn replaced soybeans in the rations containing casein (lots 4 and 5) the gains were as good as or better than those obtained with soybeans in the ration (lots 2 and 3), comparing lots 4 and 2, and 5 and 3. Dried yeast (lot 6) did not improve the basal ration materially, and yeast additions to rations carrying high and low levels of casein (lots 7 and 8) produced variable results.

In general, there was a high degree of correlation between the data obtained for swine and those obtained for rats.

CONCLUSIONS

A basal ration of corn, soybeans, and mineral is not adequate for satisfactory growth of rats under experimental conditions or for young pigs in dry lot.

The growth of pigs and rats on corn-soybean rations can be improved by the addition of casein in amounts sufficient to 2.25 or 5 per cent protein.

Although casein supplements corn alone, the addition of casein to corn-soybean rations was of no value in improving the utilization of soybeans. No evidence was obtained that casein protein supplemented soybean protein.

Cooked soybeans have a definitely superior nutritive value to raw soybeans, due to factors not apparent from these experiments. When cooked soybeans were used less feed was required to produce a unit gain in weight than when raw soybeans were used.

The protein of cooked soybeans appears to have a nutritive value somewhat less than an equivalent amount of casein when combined with corn.

The addition of 3 per cent dried yeast to rations of corn and soybeans did not improve the growth rate of the animals sufficiently to make it of economic value.

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THE EFFECT OF ENVIRONMENT ON THE NEMATODE OF THE TOMATO GALL[1]

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INTRODUCTION

Root knot has been known to occur in the United States since 1805. Neal (*11, p. 9*)[3] stated that he found it prevalent in Florida in 1876 and that "from time immemorial [it] had been dreaded as a foe to gardens and groves." The range of the disease now extends from the Atlantic coast to the Pacific. The use of the glass house in plant industry has brought the climate of the South to the Northern States, and wherever this artificial climate is maintained root knot eventually occurs.

The causal organism, a nematode (*Heterodera radicicola* (Greeff) Müll.),[4] has been extensively studied in this country by Neal (*11*), Atkinson (*1, 2*), Stone and Smith (*14*), Bessey (*3*), Godfrey and Morita (*6*), and Newhall (*12*).

The purpose of the present paper is to add to the general knowledge of the activity of the root-knot organism within the gall as influenced by temperature and moisture combined, moisture alone, desiccation, and decay of attached and detached galls.

NATURE OF THE TOMATO GALL AND ITS NEMATODE POPULATION

Nematode galls caused by *Heterodera radicicola* vary in size and form according to the number and position of female nematodes present in them. On the root system of a single badly infected tomato plant the galls may range in size from one-sixteenth of an inch to 1 inch in diameter and may be 3 inches long. The material used in the present investigation was obtained from commercial greenhouses in which tomatoes, cucumbers, and lettuce were grown.

Tomato galls have a large preponderance of cortical cells. An examination of cross and longitudinal sections shows that the pressure of these cells has altered the direction of the conducting channels of the root system, even to the breaking point. Atkinson (*1*), Stone and Smith (*14*), and Bessey (*3*), who have made particular studies of such distortions, ascribe a considerable portion of nematode injury to this disturbance, and a small amount of injury to the feeding habits of the parasite.

If a large nematode gall from a tomato plant is kept in a moist chamber for a few days the nematode population can be more easily examined. When the gall is broken open a hand lens will reveal a great many female nematodes, which appear as small, more or less

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[2] The author acknowledges his indebtedness to Prof. A. Vincent Osmun for his untiring interest in the progress of this investigation.

[3] Reference is made by number (italic) to Literature Cited p. 284.

[4] Caconema is the genus proposed by Cobb as reported by Stiles (*13, p. 118–121*) in 1924. Because the root-knot organism differs in some respects from the true Heterodera as represented by the sugar-beet nematode (*Heterodera schachtii* Schmidt), Cobb believes the genera should be separated. *H. radicicola* (Greeff) Müll. has, however, been the accepted name since 1884 (*10*). In 1872 Greeff (7) called the root-knot nematode *Anguillula radicicola* and in 1889 Neal (*11, p. 27*) called it *A. arenaria*.

opalescent, pearlish bodies. On some of these bodies there is a brownish area which, when examined under the low-power lens of the microscope, is seen to be a mass of eggs containing nematode embryos in all stages of development. Dark areas in the tissue of the gall, when examined with the low-power lens, show a mass of nematodes in all stages, ranging from the undifferentiated granular egg to a female nematode in the process of enlarging to the pyriform shape that characterizes maturity. Galls in the last stages of decay actually teem with nematode life.

Among the numerous forms of the root-knot nematode in a single tomato gall, there appears infrequently a mature female that corresponds to the brown cyst stage of the sugarbeet nematode, *Heterodera schachtii*. It contains a mass of eggs. Specimens of this form were identified by G. Steiner, senior nematologist of the United States Department of Agriculture, as females of the root-knot nematode. The existence of the cyst form has been denied by some investigators, who have made use of its apparent nonexistence in separating the two species *H. radicicola* and *H. schachtii*. Not in recent years has the subject of the cyst stage of *H. radicicola* appeared in print, yet the subject exists in a controversial state in previous literature on the subject, the cyst form being both described and denied.

It is important to state that the cyst form was found in galls from mature greenhouse plants growing in a soil that was fairly dry—so dry that the root system when pulled and shaken was left with scarcely any soil clinging to the roots. The galls were large, one-half to 1 inch in diameter, and more woody or corky than is common.

Examinations of cucumber and lettuce galls have failed to reveal the cyst form. Furthermore, the average gall on the tomato plant does not carry it. Dry soil conditions seem to be the determining factor where cyst-bearing galls are found.

COMBINED EFFECT OF TEMPERATURE AND MOISTURE ON NEMATODES

Observations in greenhouses where root knot was seriously interfering with the productivity of the tomato crop indicated that the degree of infestation might be influenced by a soil-moisture relationship. In houses facing the south, the soil, as a rule, is moister on the north half. Usually it is necessary to water more frequently on the south half, especially the spring crop. The examination of tomato roots in such houses showed a general infestation of nematodes, but the plants from the north half were less galled than those in the drier soil of the south half. Plants near a leaky water valve and a dripping pipe from an overhead watering system also showed fewer and smaller galls. As a result of these observations a series of experiments designed to yield information on the combined effect of temperature and moisture on root knot was carried out.

The range of soil temperatures employed in these experiments, 15° to 30° C., was between the minimum and maximum that would permit gall formation, and the range of moistures, 40 to 100 per cent of the moisture-holding capacity of the soil, was such as would support tomato growth. The temperatures were maintained by the constant soil-temperature apparatus. The saturated soils produced plants that compared favorably with the plants at other soil moistures.

Glass tumblers were employed as containers. Each contained 200 gm. of air-dried soil brought up to 60 per cent moisture content. Inoculations were made by mixing 5 g. of chopped nematode galls with the soil in each tumbler. For three weeks after the inoculations the tumblers were allowed to remain at greenhouse temperature. Then the soil in each was thoroughly mixed, adjusted to a specified moisture content, planted with two tomato seedlings, and placed in the temperature-control apparatus with the soil level below the level of the water bath. Table 1 gives the ranges of temperature and moisture. The experiments were allowed to run 30 days, during which time the moisture was adjusted daily by adding water equal to the loss in weight. The 30-day period was chosen because it was believed that in that time galls would have formed but probably would not have interfered seriously with the general character of the root system. However, certain discrepancies appear in Table 1 that may be due to unrecognized nematode activity. At the conclusion of the experiment, the soil was carefully washed from the roots of the plants and the better plant in each container was placed in series A and the poorer plant in series B. By the use of the scoring method suggested by Free (*4*), the root systems were compared in a manner that gave a definite score for each system within the series. Experiment 1 was conducted during January, 1926; experiment 2 was carried on from June 23 to July 24, 1926, and therefore had the better light of midsummer. The scores showing the relative effects of temperature and moisture combined are presented in Table 1.

TABLE 1.—*Effect of temperature and moisture on the ratio of galls to root systems*

[The larger values indicate proportionately fewer galls] *

Temperature (° C.)	Moisture content	Ratio of galls to root system				Distribution of galls
		Experiment No. 1		Experiment No. 2		
		Series A	Series B	Series A	Series B	
	Per cent	*Relative value*	*Relative value*	*Relative value*	*Relative value*	
15	40	65	59	50	14	Galls on main and lateral roots.
	60	43	48	10	41	Do.
	80	43	37	59	41	Galls on lateral roots.
	100			99	99	Few small galls on lateral roots.
18	40	59	71	5	14	Galls on main and lateral roots.
	60	43	48	32	55	Do.
	80	94	94	72	72	Galls on lateral roots.
	100			99	99	Small galls on lateral roots.
21	40	54	48	32	41	Galls on main and lateral roots.
	60	94	77	41	32	Do.
	80	99	94	64	72	Galls on lateral roots.
	100			99	99	Small galls on lateral roots.
24	40	6	12	14	19	Galls on main and lateral roots.
	60	37	31	46	50	Do.
	80	83	88	59	19	Do.
	100			90	90	Small galls on lateral roots.
27	40	1	1	19	14	Galls on main and lateral roots.
	60	25	25	32	50	Do.
	80	83	83	77	72	Galls on lateral roots.
	100			86	81	Do.
30	40	12	6	1	1	Galls on main and lateral roots.
	60	19	19	64	64	Do.
	80	71	77	23	46	Do.
	100			81	86	Small galls on lateral roots.

* The values are relative only within the series under which they occur. The better plants are in series A, the poorer in series B, experiment No. 1 was conducted in January, No. 2 in June and July.

Within the range of soil temperatures employed, it is apparent that there were more galls in proportion to the size of the root system at the higher temperatures, 24°, 27°, and 30° C., with no particular temperature of critical importance. At lower temperatures, 15°, 18°, and 21°, the root systems were freely galled, but much less so than those at the higher temperatures. As the optimum soil temperature for the growth of tomatoes is close to 25° (*5*, *9*), it is to be expected that root knot will be prevalent on tomato at about that temperature. The relative values in Table 1 do not change markedly with a slight increase in temperature. This finding is in agreement with that of Godfrey (*5*) who, in addition to tomato, used cucumber, tobacco, and soybeans as indicator plants. All these plants have a fairly high optimum soil temperature.

The differences in the results obtained with the different soil moistures were quite marked. Plants grown in the saturated soils (100 per cent moisture) were characterized by long slender roots burdened with very few galls. In such soils the galls were more plentiful at the higher temperatures. No plant was free of galls. In general, as the moisture content of the soil was increased, the proportion of galls decreased. There were more galls at the 60 and 80 per cent soil moistures than at the 40 per cent moisture, but the root systems of the plants at 40 per cent were so small that the plants would soon have died. The plants at 100 per cent soil moisture showed signs of spindling, but this was due to too much moisture and not to the presence of nematodes.

An examination of the data in Table 1 showing the distribution of galls on the roots indicates that the main root of the tomato seedling becomes burdened with galls at the lower moisture contents and also at the higher temperatures. At the higher moisture contents the galls are, for the most part, on the side roots. Infection on the main root interferes more seriously with the growth of the plant than does the presence of galls on the lateral roots.

As previously stated, in the soils of highest moisture content (100 per cent) the roots were very long and slender and such roots had few galls, if any. It therefore appeared possible that the size of the root might aid or prevent invasion by the nematode. It is commonly understood that the invading nematode attacks the loosely massed cells of the meristematic tissue of the root tip, and so gains entrance to the root. By employing a root cage with tomatoes growing in a nematode-infested soil, a horizontal microscope, and a strong light focused on root tips, the writer was able to make direct observations of a nematode in the process of invading a root. The worm had worked its way in at the root tip and upward through the meristematic region, the undulations of its body aiding in the observation. A small portion of the posterior end had apparently not fully entered the root tip, for it was in the place normally occupied by the root cap. A measurement with an ocular micrometer determined the length of the organism in the observed stage as 933μ. (Fig. 1.)

Beaded arrangements of small galls seem to confirm a theory that the nematode does not stop after forcing its way into the root tip, but travels into the tissue that is developing the central cylinder to avoid being carried forward by the progressive growing point of the root tip.

EFFECT OF MOISTURE ON NEMATODES

In order to obtain more definite information concerning the effect of moisture on nematodes in galls, fresh tomato galls as large as one-half inch in diameter were mixed with 400 g. of air-dry soil and kept in pint glass jars. To the soil was added sufficient water to produce the range of soil moistures shown in Table 2. The jars were loosely capped with paper, which was held in place by rubber bands. This helped to maintain a constant soil moisture. The jars were weighed every 48 hours and brought up to weight when necessary. At the end of the 31-day period the soils were mixed with enough nematode-free soil (steamed soil) to fill a 6-inch pot. The pots were then planted with tomato seed and four weeks later the seedlings were examined, with a final examination 15 weeks after the seed was planted. The results, which are presented in Table 2, show that the nematodes survived at all moistures except at 0 per cent, i. e., in the air-dry soil. Even in soils too deficient in moisture (10 and 30 per cent) to sustain crop plants, the nematodes were able to survive.

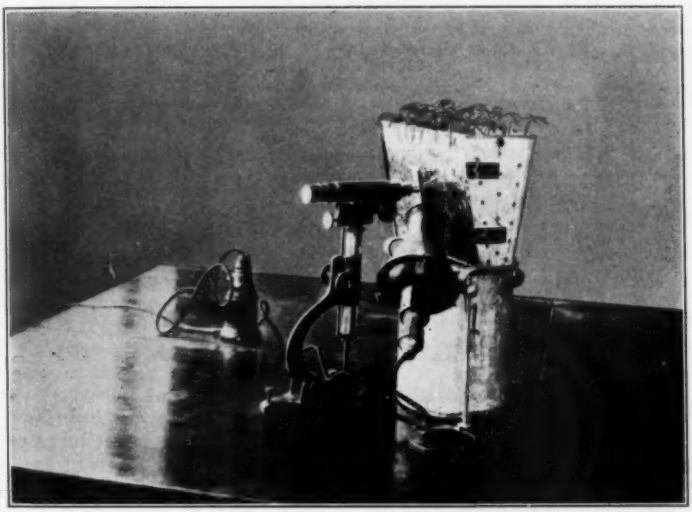

FIGURE 1.—Apparatus used for observing root invasion by nematodes

TABLE 2.—*Effect of soil moisture upon the survival of the nematode in galls*

Moisture in soil (per cent) [c]	First examination [a]		Second examination [b]		Moisture in soil (per cent) [c]	First examination [a]		Second examination [b]	
	Plants nematode free	Plants nematode infected	Plants nematode free	Plants nematode infected		Plants nematode free	Plants nematode infected	Plants nematode free	Plants nematode infected
	Number	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*
0	5	0	3	0	70	0	5	0	3
10	0	5	1	2	80	0	5	0	3
30	2	3	0	3	90	0	5	0	3
50	3	2	0	3	100	0	5	1	2
60	1	4	0	3					

[a] 5 plants examined 4 weeks after seeding.
[b] 3 plants examined 15 weeks after seeding.
[c] Percentage of saturation.

EFFECT ON NEMATODES OF FLOODING A GALL-INFESTED SOIL

After it had been determined that a saturated soil (100 per cent moisture) will support nematode life and allow invasion to take place, it was decided to carry the study of moisture relationships one step further and include a flooded soil.

For this study galls were buried in sand in glass tumblers and the water level maintained at least one-half inch above the surface of the sand for various periods, as shown in Table 3. At the end of each period a tumbler was removed and its contents mixed with sterile soil in a 6-inch pot, which was then planted with tomato seed. A pot containing sterile soil was likewise planted with tomato seed and placed in the group of pots as a check on contamination. At the close of the experiment no galls were found in the check pot, but galls were present in all other pots. It is therefore apparent that even 28 days of continuous flooding is not sufficient to destroy the nematodes in gall-infested soil.

TABLE 3.—*Effect of flooding upon the survival of nematode in galls*

Period flooded (days)	Days from seed	First examination		Final examination		Period flooded (days)	Days from seed	First examination		Final examination	
		Plants nematode free	Plants nematode infected	Plants nematode free	Plants nematode infected			Plants nematode free	Plants nematode infected	Plants nematode free	Plants nematode infected
		Number	*Number*	*Number*	*Number*			*Number*	*Number*	*Number*	*Number*
0	25	4	6	0	3	6	19	0	10	0	3
1	24	2	3	0	3	7	18	0	10	0	3
2	23	0	10	0	3	10	25	0	10	0	3
3	22	0	10	0	3	14	24	0	4	0	3
4	21	0	10	0	3	21	42	7	19	0	3
5	20	0	10	0	3	28	35	20	9	0	3
Check	36	20	0	3	0						

EFFECT ON NEMATODES OF DESICCATION IN SOIL

Although several investigators have reported that nematodes can be destroyed by the process of gradual desiccation, no information was available as to just what is the maximum period that large galls will support nematode life in the desiccating atmosphere of an air-dry soil. Accordingly, a series of experiments designed to throw light on this point was conducted.

In a preliminary experiment, large galls (about three-fourths inch in diameter) were introduced into 6-inch pots of air-dry soil. After 2, 3, and 4 weeks these pots, in replicates of five, were moistened and seeded with tomato. The 3-week and 4-week desiccation periods destroyed all nematode life, but the 2-week period did not.

For the next two experiments galls one-half inch in diameter were employed. Six-inch pots of air-dry soil were divided into groups which were kept dry for various periods, as indicated in Table 4, and then moistened and planted with tomato seed. In the check pots, however, the soil was moistened immediately after the galls were introduced into the air-dry soil. All groups were in replicates of five. In experiment 1, positive infection was obtained in each of the check pots. The second group (galls in air-dry soil for two weeks) gave

negative results. Unfortunately, it was not anticipated that eradication might be secured in less than two weeks, and observations were unduly delayed. All remaining groups gave negative results, as shown in Table 4.

TABLE 4.—*Effect of desiccation in soil upon the survival of nematodes in galls*

Period in air-dry soil (weeks)	Experiment No. 1, Dec. 10, 1926–Apr. 28, 1927					Experiment No. 2, Aug. 23–Dec. 16, 1927				
	Nematode galls * in pot No.—					Nematodes galls * in pot No.—				
	1	2	3	4	5	1	2	3	4	5
Check	+	+	+	+	+	+	+	+	+	+
½	—	—	—	—	—	+	+	+	+	+
1	—	—	—	—	—	+	+	+	+	+
1½	—	—	—	—	—	0	+	+	+	+
2	0	0	0	0	0	0	0	0	0	0
2½	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
3½	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
4½	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
5½	0	0	0	0	0	—	—	—	—	—

* The sign (+) indicates the presence of nematode galls; the dash (—) infers that no examination was made for the indicated period; and 0 indicates the absence of nematodes.

The experiment was repeated with galls of the ½-inch size and provision made to reduce all periods to multiples of ½-week duration. In the second experiment, eradication was effected in more than one and one-half weeks and less than two.

It is apparent that the ¾-inch galls used in the preliminary experiment required a longer period of desiccation than the ½-inch galls employed in the two experiments shown in Table 4. From these results it is obvious that the length of the survival period is influenced by the size of the galls as well as by the length of time that the galls remain in the air-dry soil.

DECAY OF GALLS, ATTACHED AND DETACHED, IN SOILS OF DIFFERENT MOISTURE CONTENT

A study of the influence of environment on the nematode organism is more involved under natural conditions than it is in the laboratory. Particularly is this true when the galls are large.

In addition to harboring a dormant phase of nematode activity, the galls certainly must act as protective agents to their nematode population. A decayed gall has less protective action than a fresh one. A gall attached to a living root may persist in the fresh stage longer than does a detached gall. Even though these galls remain in the soil for a considerable time, they may resist decay owing to the antiseptic properties of living cells, which are kept alive by the root of which they are a part. That such a relation exists is borne out by the following experiment in which the decay of galls, attached and detached, was studied in soils holding different percentages of moisture.

Equal weights (10 pounds) of air-dry soil were placed in containers divided into five series of five pots each at moisture contents of 0, 40, 60, 80, and 100 per cent of the water-retaining capacity of the

soil. The water-holding capacity of the soil as determined by the method of Hilgard (*8, p. 209*) was 53 per cent of its air-dry weight.

Growing plants galled by nematodes were planted in each container. The soil in the containers was maintained at 70 per cent moisture until all plants were well established. This took about three weeks. The low-moisture containers were reduced to their 0 and 40 per cent values in about 5 days. All weights were maintained by frequent weighing.

When all pots were adjusted to the proper moisture percentage detached galls from other plants were placed in cages of window screening and deposited in each container with some of the soil to insure the same environment as the attached galls. The living plants were then cut at the surface of the ground without disturbing the root system. At intervals of a week one container from each series was removed and the attached and detached galls were examined. The results are recorded in Table 5.

TABLE 5.—*Effect of various soil moistures on nematode galls from tomato; attached and detached galls*

Period galls were in soil (weeks)	Attached galls	Detached galls
SERIES A, 0 PER CENT MOISTURE CONTENT		
1	Turgid, not shrunken, galls apparently perfect.	Slightly shriveled, inclined to be brittle.
2	Indications of shriveling, but on the whole turgid.	Very shriveled and dry, not brittle.
3	Smaller galls shriveled, larger galls hard and turgid.	Very shriveled, brittle.
4	Shriveled, soft, not brittle	Do.
5	Shriveled, punky, not decayed, not brittle	Do.
SERIES B, 40 PER CENT MOISTURE CONTENT		
1	Turgid, unchanged	Softening, inclined to be spongy.
2	Slightly shriveled, slight decay	Soft decay, epidermis easily sloughs.
3	Soft, punky, dry and fibrous	Very fibrous, not much remaining.
4	Very punky, reduced in size, epidermis easily sloughs.	Nothing left but tough central cylinder.
5	Galls whole, epidermis easily sloughs, slight decay.	Only central cylinder remains, not hard.
SERIES C, 60 PER CENT MOISTURE CONTENT		
1	Turgid, unchanged	Very soft, inclined to be fibrous.
2	Slightly shriveled, slight decay	Nothing left but central cylinder which is hard.
3	Punky, sloughs to a hard central cylinder	Soft central cylinder.
4	Epidermis whole, tissues to central cylinder missing, central cylinder soft.	Central cylinder hard.
5	Epidermis whole, tissue beneath gone as far as hard central cylinder.	Central cylinder nearly destroyed, very fibrous and soft.
SERIES D, 80 PER CENT MOISTURE CONTENT		
1	Turgid, unchanged	Very soft, mushy.
2	Galls whole, mushy	Nothing left but soft central cylinder.
3	Soft, epidermis tough, hard central cylinder.	Soft central cylinder.
4	Epidermis soft, somewhat decayed, inner tissues missing, central cylinder soft.	Do.
5	Epidermis whole and fairly tough, tissues missing to central cylinder which was pliable.	Central cylinder whole and brittle.

TABLE 5.—*Effect of various soil moistures on nematode galls from tomato; attached and detached galls*—Continued

SERIES E, 100 PER CENT MOISTURE CONTENT

Period galls were in soil (weeks)	Attached galls	Detached galls
1	Soft, gall intact	Very soft, central cylinder exposed due to sloughing of tissues surrounding it.
2	Galls whole, very mushy	Galls whole but all decayed under epidermis, central cylinder hard.
3	Epidermis whole and tough, fleshy tissues all gone, hard central cylinder.	Epidermis whole and soft, hard central cylinder.
4	Epidermis whole, tissues missing, central cylinder soft.	Epidermis whole, tissues missing, central cylinder tough and hard but not brittle.
5	Epidermis whole and tough, tissues missing to pliable central cylinder.	Epidermis whole and tough, tissues missing to brittle central cylinder.

At every moisture content, the attached galls were less susceptible than the detached to the natural forces of decomposition. As the moisture content of the soil increased, the process of decay was hastened in both the attached and detached galls. There was no decay in the containers entirely devoid of soil moisture. The galls at 0 per cent moisture gradually shriveled; the detached galls lost practically all their moisture and became brittle. At the end of the first week the attached galls were turgid at all moisture contents except at 100 per cent. In this container the galls were still intact, but their softness was evidence that the cells were ready to collapse. The detached galls in the same container were at this time (after one week) so far gone that the central cylinder was exposed, with the surrounding tissue dropping away.

A comparison of the progress of decay in the soils of different moisture content indicates that decay in both attached and detached galls at 100 per cent was 1 week ahead of that in galls at 60 and 80 per cent and 2 weeks ahead of that in galls at 40 per cent. However, when the rates of decay of attached and detached galls are compared, it is evident that the difference in rate of decay decreases with increase in moisture content of the soil. At 100 per cent moisture content, the attached galls lagged about 1 week behind the detached in the process of decay; at 80 per cent, the lag was 2 weeks; at 40 and 60 per cent, 3 weeks; and at 0 per cent, 5 weeks or more.

SUMMARY

This paper presents a study of the effect of environment on the activity of the root-knot nematode, *Heterodera radicicola* (Greeff) Müll., of the tomato gall.

The nematode gall of the tomato plant is well suited as material for environmental investigations. It offers substantial protection to its nematode population because of the resistant root-covering tissue.

All stages in the life cycle of the nematode may be found in a decaying gall. Among these, there was found infrequently a female nematode, which contained a mass of eggs. It was cystlike, and corresponds to the brown cyst form of the sugar-beet nematode, *Heterodera schachtii* Schmidt. This particular cystlike form was not found in galls from cucumber or lettuce plants, and only on tomato plants that were growing in a relatively dry soil.

The optimum range of soil temperature for tomato, 25° to 30° C., is also the optimum range for nematode activity. A low soil-moisture content, 40 per cent, combined with high temperatures, 24° to 30°, increased the number of galls in proportion to the size of the root system. Plants grown in a soil saturated with water had very few galls, especially at the lower temperatures, 15° to 21°. Such plants have long slender roots.

An apparatus is described for observing the invasion of a root by a nematode. The suggestion is made that the size of a root may aid or prevent invasion by nematodes.

The nematode in a detached gall may survive for one month or longer in soils ranging in moisture content from 10 to 100 per cent. The organism did not survive this period in an air-dry soil.

Flooding a gall-infested soil for 28 days did not eradicate the nematode.

Galls in an air-dry soil did not protect nematode life as long as 14 days, but did protect it as long as 10 days.

A detached gall decays more rapidly than a gall attached to a root system in situ. The rate of decay of attached and detached galls increases with an increase of moisture in the soil. The rate of decay of an attached gall lags about three weeks behind that of a detached gall in a normally moist soil, that is, one containing approximately 60 per cent moisture.

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